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I. "On the Colouring and Extractive Matters of Urine."—Part I.

By EDWARD SCHUNCK, F.R.S. Received June 29, 1865*.

Of all the animal secretions urine is undoubtedly one of the most important. Its varying properties, in health as well as in disease, the frequency with which it is emitted, and the consequent facility with which it may be submitted to examination, render it invaluable to the physiologist and pathologist as a means of throwing light on the processes, either healthy or morbid, going on within the body. Its study has therefore engaged the attention of physicians since the earliest times, and of chemists from the period when chemical analysis was first employed in the examination of natural objects. Notwithstanding the labour bestowed on the subject by many eminent men during the past sixty years, it is still, however, far from being exhausted. There are, indeed, portions of the chemistry of urine concerning which our ignorance is almost complete. It is one of these obscurer parts of the subject that I have endeavoured to clear up, and I hope to succeed in showing that I have added at least a few facts to the sum of our previous knowledge.

Of all the properties of urine none is more obvious, even to the ordinary observer, than its colour. The variations in tint which it exhibits at different times are striking, even to the unpractised eye, and they sometimes serve as important indications to the physician. Nevertheless concerning the chemical nature of the substances to which its colour is due very little is known. Our ignorance on this subject may be ascribed to various causes. In the first place, some of these substances occur in the urine only occasionally, and in very minute quantities, so that the preparation of a quantity sufficient for chemical examination becomes difficult and even impossible, especially when the urine containing them is not abundant. Secondly, it has been found that some of them are very easily decomposed, so much so that the mere heat required for the evaporation of the urine seems to be sufficient to effect a change in their properties and composition. It therefore becomes doubtful, after a long process has been gone through for the purpose of separating any colouring-matter from the other constituents of the urine (a process in which, perhaps, strong chemical reagents have been employed), whether the substance procured was originally contained as such in the urine, or is not rather a product resulting from the decomposition of some other substance or substances. Thirdly, several of the bodies colouring the urine possess very few characteristic properties. They are amorphous and syrup-like, and they retain water with so much pertinacity that on attempting to dry them they undergo decomposition. Neither their compounds nor their products of decomposition exhibit any distinguishing characteristics. They belong to a class on which, for want of a better, the name extractive matter has been

* Read January 11, 1866: see Abstract, vol. xv. p. 1.

conferred. With some chemists, to call a body an extractive matter is to place it among a class which is held to be unworthy of minute examination. To others the name extractive matter is merely a convenient word for a mixture, sometimes occurring in nature, of certain definite, perhaps even crystallized substances, which, by appropriate means, may be resolved into its constituents, and thus be made to disappear entirely from the list of definite chemical bodies. As regards the extractive matter of urine, this view may to some extent be justified, when we recollect that from what was considered to be extractive matter sixty years ago, such well-characterized substances as urea, hippuric acid, and creatine have been successively eliminated; and it is therefore natural to expect that by further research it will be found to contain others of the same nature. I believe this view to be erroneous; and I shall succeed, I hope, in showing that, after having removed from the extractive matter of urine everything which can assume a definite form, there remains a residuum which cannot be further resolved without decomposition. Still, any one holding this view is not likely to undertake the investigation of extractive matters as such, unless it be for the purpose of obtaining something which may be supposed to be contained in them. Lastly, the properties of these colouring and extractive matters, however important they may be to the physiologist and pathologist, present so little that is interesting to the chemist, that the latter would probably not occupy himself with their examination unless for some particular purpose. For myself, I frankly confess that, had I not had a special object in view, this investigation would not have been undertaken. The information for the sake of which it was commenced having been obtained, I should then have abandoned all further inquiry, had I not found reason to suppose, in the course of my experiments, that a more extended investigation would lead to results interesting from a physiological point of view. My endeavours have, I think, been attended with some measure of success; and should physiologists, on becoming acquainted with the results, be of the same opinion, my labour will not have been quite in vain.

The colouring-matters which occur in, or have been obtained from, urine may be divided into three classes, viz. :—

1st. Those which are only found occasionally in it, in consequence either of disease or of some abnormal state of the system.

2ndly. Those which are produced by spontaneous decomposition, or by the action of reagents on substances, either coloured or colourless, pre-existing in the urine.

3rdly. The colouring-matter or matters occurring in normal urine, and to which its usual colour is due.

A few remarks on the present state of our knowledge on these three classes of pigments, as derived from the labours of my predecessors as well as my own, may not be out of place.

I. The abnormal colouring-matters, which are found ready formed in the urine, may either be peculiar to the secretion, or their presence may be

due to an admixture of blood, bile, or milk, causing the urine to assume various shades of red, green, or white. The latter, as well as those which make their appearance in consequence of the administration of certain drugs, I leave entirely out of consideration. The others, or those peculiar to urine, may be conveniently divided, according to their colour, into three classes, viz., blue, purple or red, and black or brown colouring-matters.

The appearance of a blue colouring-matter in urine has been frequently observed both in ancient and modern times. Cases of its occurrence have been recorded by Janus Plancus*, Delens†, Spangenberg‡, Prout§, Simon||, Braconnot¶, Julia-Fontenelle**, Cantu††, Reinsch‡‡, and Du Ménil§§. In all these cases the urine yielded a deposit varying in colour from slate-grey to light blue, or even dark blue, consisting of a blue colouring-matter generally mixed with earthy phosphates. The colouring-matter, after being separated from the impurities with which it was contaminated, was in most of these cases found to have so many properties in common with indigo-blue that several observers, such as Prout and Simon, seemed to have no doubts concerning its identity with the latter. It was, for instance, insoluble in water, but somewhat soluble in alcohol and ether. It was destroyed by nitric acid, but was not affected by other acids, except concentrated sulphuric acid, with which it yielded a blue solution. It was not dissolved by alkalis, except when some reducing agent, such as grape-sugar, was added at the same time. It then dissolved, but was again deposited from the solution on exposure to the air. On being heated, it yielded a violet-coloured vapour. Julia-Fontenelle and Cantu, however, maintain that the colour in the cases examined by them was due to prussian blue; and Angelini||| suggests that it may possibly be ascribed to phosphate of iron. Lastly, Braconnot has described a blue colouring-matter obtained from urine, which, if his observations are correct, differs entirely from all other pigments derived from the same source. Like indigo-blue it was insoluble in water and alkalis, and only slightly soluble in boiling alcohol; but, on the other hand, it dissolved with ease in dilute acids, forming solutions of a brownish-yellow colour, which, on the addition of an excess of acid, assumed a brilliant red tint. From its solution in acid it was precipitated by alkalis and alkaline earths. To this colouring-matter Braconnot gave the name of *cyanourine*. Since his time, how-

* Commentarii Instituti Bononiensi ad ann. 1767.

† Schweigger's Journal f. Physik u. Chemie, B. xxiii. S. 262.

‡ Ibid. B. xlvii. S. 487.

§ On Stomach and Renal Diseases, 5th ed., p. 567.

|| Simon's Animal Chemistry, translated by Day, vol. ii. pp. 274 & 327.

¶ Annales de Chimie et de Physique, t. xxix. p. 252.

** Archives générales de Médecine, t. ii. p. 104.

†† Mémoires de l'Académie Royal de Turin.

‡‡ Jahrbuch f. Pract. Pharm., B. viii. S. 93.

§§ Archiv d. Pharm., B. xxxix. S. 48.

||| Giorn. di Fisica, Dec. II. t. viii. (1825).

ever, no one has obtained any substance from urine having exactly the same properties. The urines which deposit the blue colouring-matter are not found to exhibit any peculiarities in other respects, nor does the deposit appear to be characteristic of any peculiar class of diseases. It seems occasionally even to make its appearance during a state of perfect health. Sometimes the deposit seems to contain also another colouring-matter, more easily soluble in alcohol and ether, to which it communicates a fine purple colour.

The deposits of urate of ammonia and urate of soda, which are formed in urine during fever and other diseases, are always found to exhibit different shades of red, varying from pink to carmine. To what this colour is to be attributed has not yet been satisfactorily ascertained. Proust*, who was the first chemist to examine these deposits, thought that he had discovered in them a peculiar coloured acid, which he called *rosacic acid*. It is almost certain, however, that the acid properties of this body were due to an admixture of uric acid. Indeed, Vauquelin, after an examination of this so-called acid, arrived at the conclusion that it was a compound of ordinary uric acid with an intensely red colouring-matter. Vogel†, it is true, professed to have obtained pure rosacic acid by treating the crude deposits with boiling alcohol, but as, according to him, it is converted with great facility into uric acid by the action of sulphuric and nitric acid, it is very probable that his substance still contained some of the latter acid, and that the supposed conversion consisted merely in a destruction of the organic colouring-matter. Fromherz and Gugert‡ also made some experiments with these red deposits, from which they infer that rosacic acid consists of a neutral, red extractive colouring-matter, mixed with uric acid and urate of soda, which may be separated by treating the mixture first with water and then with warm alcohol, which dissolves the colouring-matter. The latter, after being thus separated from the other constituents, no longer yields uric acid. Prout§ suggested that the colour of the red deposits might be due to purpurate of ammonia, the purpuric acid being formed in some unexplained manner by the action of nitric acid on a portion of the uric acid contained in them. To this it was objected by Berzelius|| that purpurate of ammonia is insoluble in alcohol. He mixed urate of ammonia with a solution of a purpurate in acetic acid, which does not destroy the colour, and he observed that the precipitated uric acid acquired a pale pink colour closely resembling that of the urinary deposits; but this colour was not removed by boiling alcohol, in which, on the contrary, the colouring-matter of the red deposits is easily soluble. Duvernoy¶ asserts that he succeeded in preparing a colouring-matter identical with that of the red deposits by evaporating ordinary

* Annales de Chimie, t. xxxvi. p. 265. † Ibid. t. xevi. p. 306.

‡ Schweigger's Journal, B. I. S. 199. § Annals of Philosophy, vol. xv. p. 155.

|| Lehrbuch der Chemie, B. ix. S. 421.

¶ Untersuchungen über den menschlichen Urin. Stuttgart, 1835.

healthy urine to one-third or one-fourth of its volume, adding a little nitric acid, allowing it to stand for a day, during which time the colour of the liquid changed from yellowish brown to dark red, and then mixing with a solution of urate of potash. A precipitate was thereby formed of uric acid, having the same red colour as the natural red deposits, from which the red colouring-matter could be extracted by means of alcohol. Recent observers have given names to this colouring-matter, such as *uroerythrine* and *purpurine*, without, however, adding anything of importance to our knowledge of its properties. The method adopted by them for its preparation is essentially the same as that first suggested by the earlier chemists. The deposits containing it are washed with water, and then digested with warm absolute alcohol, which takes up the colouring-matter and, after filtration and evaporation at a temperature not exceeding 50°C., leaves it in the form of a red amorphous residue. It cannot be obtained by evaporating the urine containing it; but on dissolving white and pure urate of ammonia in urine (which by its pink or purple colour indicates the presence of purpurine), the salt is precipitated, on cooling, deeply coloured, and yields the colouring-matter on being treated in the way just described. It is not improbable that this purpurine and the blue colouring-matter just referred to may stand in some relation to one another. An observation made by Angelini* seems to favour this view. This chemist, being desirous of examining the pink deposit which was being formed in his own urine during an attack of fever, had it collected and laid aside; but being unable, from the state of his health, to examine it at once, it remained for some days exposed to the atmosphere, and during this time the pink colour changed in many places into blue. On leaving it to stand for some time longer, the blue tint did not spread further, but the spots became darker in colour.

Instances of black urine are even of rarer occurrence than those of urine coloured blue. Indeed in many cases the black colour seems to have been due to red or purple pigments, which communicated to the urine so deep a tint as to make it appear black. Dulk, for instance, obtained from a black urine a substance of the same colour containing iron, which Berzelius† with some reason suspected to be merely hematine. In the case described by Marcet‡, the urine appears to have been purple, or purplish-brown in the first instance, and to have become black on standing. It contained no red blood-globules and no trace of iron, and yielded no coloured deposit on standing for a length of time, the colouring-matter being kept in solution by the alkali, which was always present in excess. This colouring-matter was examined by Prout, who gave it the name of *melanic acid*. It was precipitated from the urine by means of acids in black flocks, which were found to be nearly insoluble in water and alcohol, but readily soluble in caustic and carbonated alkalies, the solutions being of a very dark colour.

* Giorn. di Fisica, Dec. II. t. viii. (1825).

† Jahresbericht, 20ter Jahrg., S. 576.

‡ Medico-Chirurg. Transactions, 1822.

The solution in ammonia gave copious brown precipitates, with metallic salts. Marcet concludes from the experiments of Prout that this so-called acid bears a close analogy to the products derived from uric acid; but Berzelius remarks that it strongly resembles the black pulverulent substance, insoluble in alcohol, which is formed by the action of concentrated acids on the extractive matters of urine. By heating the urine yielding cyanourine, after separation of the latter by filtration, Braconnot obtained a black sediment which he called *melanourine*. I should at once have assumed that this substance was identical with Prout's melanic acid, if Braconnot had not stated that his black pigment was soluble in weak acids and insoluble in alkalies, whilst the behaviour of melanic acid to acids and alkalies is exactly the reverse. Considering the facility with which the ordinary extractive matters of urine are decomposed, yielding products insoluble in water of a black or brown colour, it is surprising that urines containing these bodies ready formed should not more frequently be met with in cases of disease. It is not improbable, however, that the dark-brown colour of some urinary calculi may be owing to one or the other of these bodies.

II. The second class of urinary colouring-matters comprises those which are formed from urine by artificial means, and consequently do not exist ready formed in the secretion. These may also be classified according to colour, those which have hitherto been observed being either blue, red, or brown.

I believe that Heller* was the first to obtain artificially from urine colouring-matters of a pure blue or red tint. He states, in his first memoir on the subject, that in some diseases the urine contains a notable quantity of a body of a light yellow colour, and easily soluble in water, which he calls *uroxanthine*. When urine containing this body is exposed to oxidizing agencies, such as nitric acid, or even atmospheric air, it deposits a dark-coloured sediment, consisting of a blue and a red colouring-matter, named by him respectively *uroglaucine* and *urorhodine*. The former, after being purified, appears in small groups of crystals of a dark-blue colour, which are insoluble in water, as well as in cold alcohol and ether, but soluble in boiling alcohol. Urorhodine, according to Heller, is formed by a lower degree of oxidation than uroglaucine. It is easily soluble in cold alcohol or ether, to which it communicates a splendid crimson colour, and is always amorphous and apparently of a resinous nature. Uroxanthine, the body from which these colouring-matters are derived, and which, according to Heller, is itself probably derived from urea, is also contained in small quantities in normal urine. Braconnot's cyanourine is, in Heller's opinion, a mixture of uroglaucine and urorhodine. In two subsequent memoirs† Heller communicated some further details on the preparation of these colouring-matters from urine, and on their occurrence in a urinary calculus, without, however, adding any new facts to those previously known regard-

* Heller's Archiv, 1845, S. 161.

† Ibid. 1846, S. 19, 536.

ing their chemical or physical properties. The experiments of Alois Martin *, the results of which were made known soon after those of Heller, led to the same conclusion, viz., that in some diseases the urine on being mixed with mineral acids deposits in considerable quantity a dark-coloured sediment, consisting of two colouring-matters, one of which is blue, the other red. Regarding the former, which he calls *urokyanine*, Martin states that it is insoluble in water and caustic alkalies, but soluble in alcohol and ether, that it is dissolved by concentrated sulphuric acid, the solution becoming blue on dilution with water, and that when heated it yields violet-coloured fumes like those of iodine. Although these observations, however incomplete, were no doubt correct, very little importance was attached to them by chemists in general, and their accuracy was even questioned by some. Berzelius characterizes Heller's statements as uncertain and unsatisfactory. Lehmann says, "Heller's experiments were so incomplete that the very existence of such pigments as uroxanthine and urorhodine is still doubtful." Golding Bird was of opinion that Heller had described as crystals of uroglauine uric acid merely tinted by the changed colouring-matter, and he adds, "This error is an important one, and throws much doubt on many of his conclusions." When a few very simple experiments would have sufficed to prove the accuracy of the observations referred to, or to have shown in what respect they were erroneous, such criticisms as these can hardly be considered fair; and I think that Heller's claims as the discoverer of the artificial formation from urine of a blue and a red colouring-matter of definite character cannot be contested. Golding Bird certainly claims to have been the first to observe the formation of a red or pink colouring-matter, supposed by him to be identical with that of the so-called pink deposits, by the action of hydrochloric acid on healthy urine; but, without deciding the question of priority, I will merely remark that his experiments must have been of a superficial character, or the simultaneous formation of a blue colouring-matter would hardly have escaped his notice. On the other hand, when it is considered that the blue pigment occasionally deposited from urine had, as mentioned above, been proved to be indigo-blue by several of the earlier observers, and that at the time when Heller and Martin gave an account of their experiments the properties and products of decomposition of this colouring-matter were well known, it is surprising that these chemists should not have suspected the identity of uroglauine, urokyanine, and indigo-blue. A few comparative experiments would have proved their identity, and have thus led to the discovery of one of the most important and interesting facts connected with the chemistry of this subject. How far Heller was from understanding the true nature of his blue colouring-matter will be seen by the following extract from his last memoir. He says, "If a pale yellow urine, rich in uroxanthine, either originally alkaline or alkaline through standing, be kept in a well-corked flask, the violet-coloured sub-

* Heller's Archiv, 1846, S. 191, 287.

stance separates, mostly at the surface, but partly at the bottom. If the flask, while still closed, be shaken, scarcely any change of colour takes place; but if it be shaken after the stopper has been removed and air admitted, the urine becomes, by shaking with the air, more or less green, often very beautifully grass-green. On standing it again becomes pale, and these appearances may be repeated at pleasure with urine that has been kept for months in a flask. This phenomenon, viz., that a strongly alkaline urine containing the mixture of colouring-matters only becomes green by contact with air and not as long as the vessel is closed, is one the cause of which I have not as yet been able to ascertain." Any one acquainted with the properties of indigo-blue would, however, have understood the matter at once. By the combined action of the alkali and the deoxidizing matters contained in the urine, the indigo-blue in Heller's experiment was reduced and dissolved, forming a true indigo-vat, and on admitting air it was reoxidized and precipitated, to be dissolved again when the vessel was closed. Several years later H. v. Sicherer* obtained from a specimen of morbid urine, by the action of strong acids, a blue deposit, the properties of which he found to be those of indigo-blue.

Heller's experiments were followed, after an interval of some years, by those of Hassall†, who observed the formation of a blue colouring-matter on allowing urine from disease to stand for some time exposed to the air. The colouring-matter was mixed with phosphates, mucus, and other impurities; but after the latter had been, as far as possible, removed, it was found to consist of indigo-blue. Hassall inferred from his experiments that the occurrence of this substance in the urine is strictly pathological. "We should be led," he says, "to look for its occurrence in the urine in all those cases of functional derangement of any kind in which any impediment exists to decarbonization, as is the case especially in most diseases of the organs of respiration. . . . It does not appear that, by any treatment of the urine with reagents, indigo can be developed in healthy urine at will. I have made several attempts with this view, but without obtaining any definite result." This opinion proved, however, to be erroneous.

This subject was next taken up by myself‡. My experiments on the formation of indigo-blue in plants yielding that colouring-matter led to the conclusion that these plants contain a peculiar substance, belonging to the class of glucosides, which I named *indican*. As this substance is easily soluble in water, alcohol, and ether, and yields, by decomposition with acids, indigo-blue and sugar, I thought it probable that the formation of indigo-blue in urine might be due to the presence of a similar body in the secretion. This supposition was found to be correct. Not being able to procure specimens of morbid urine such as would be likely to yield the colouring-matter, I was compelled to employ healthy urine; but after de-

* *Annalen der Chem. und Pharm.*, B. xc. S. 120.

† *Philosophical Transactions*, 1854, p. 297.

‡ *Memoirs of the Literary and Philosophical Society of Manchester*, vol. xiv. p. 239.

prising the latter of the greatest part of the ordinary extractive matter by precipitation with basic acetate of lead, then adding ammonia to the filtered liquid, and acting on the precipitate produced by ammonia with sulphuric or hydrochloric acid, I succeeded in almost every instance in obtaining a small quantity of a colouring-matter, which I had no difficulty in identifying as indigo-blue. The cases in which this did not occur were so few and exceptional that I was led to conclude that indican, or some substance closely resembling it, is a normal constituent of healthy urine, and that it is only the presence of an excess of this, just as of any other of its usual constituents, that can be considered a symptom of disease. The blue colouring-matter was generally accompanied by another, which dissolved in alcohol with a fine purple colour, and which I consider to be identical with Heller's urorhodine. As the indican of plants always yields by decomposition indigo-red as well as indigo-blue, I think it not improbable that this red pigment from urine may consist of indigo-red; but from the difficulty experienced in purifying it, and the very minute quantities which are obtained, this cannot easily be proved. The urine of the horse and the cow yielded the same colouring-matters even in greater abundance than human urine. My experiments have been confirmed by Carter* and others; and it is now, I believe, generally admitted that they afford a means of explaining the formation of the abnormal colouring-matters of the urine, and may even throw some light on the processes of decomposition which the proteine substances undergo in the system. In order to prove the complete identity of Heller's uroglaucone with indigo-blue, Kletzensky† prepared a large quantity of uroglaucone, and ascertained that its properties and composition are those of indigo-blue, and he accordingly ascribes to Heller the discovery of indigo-blue in urine. I believe, however, that Heller's claims on this field of research cannot be allowed to extend so far. What I think must be conceded to him is, as I stated above, the discovery of a mode of obtaining a blue and a red colouring-matter from urine by artificial means.

The formation of brown colouring-matters by the action of acids on urine was first observed by Proust‡. Having evaporated fresh urine to a syrup, in order to separate the greatest part of its salts, he added concentrated sulphuric acid to it, and then submitted the liquid to distillation. The distillate contained a large quantity of acetic acid and a little benzoic acid, while the liquid deposited a brown mass of the consistence of pitch, which increased in quantity as the distillation proceeded. This mass consisted chiefly of a resinous body, which he found to be insoluble in water, but easily soluble in alcohol and alkaline liquids. In consistence, colour, and smell it resembled castoreum, and it had a sharp, bitter taste like that of arum-root. Proust believed it to be the substance to which the colour

* Edinburgh Medical Journal, August 1859.

† Schmidt's *Jahrbücher d. Medicin*, B. civ. S. 36.

‡ *Annales de Chimie*, t. xxxvi. p. 274; and *Annales de Chim. et de Phys.* t. xiv. p. 262.

as well as the peculiar odour and taste of urine are due, and he called it the *resin of urine*. The deposit formed in the boiling liquid contained also a black pulverulent body, which he found to be insoluble in water and alcohol, but soluble in alkalies, forming with the latter dark-brown solutions, from which it was precipitated by acids in thick black flocks. When dry, it had a shining appearance resembling that of broken asphalt. Proust called this the *peculiar black substance* from urine; and after some speculations on its nature and origin, he says that probably at some future time the relation, at present quite unknown, in which it stands to other bodies will be discovered. On repeating Proust's experiments, Berzelius obtained nearly the same results; but he was of opinion that these substances are not contained as such in the urine, as Proust had supposed, but are formed by the action of acids on the extractive matters of urine. In this opinion I entirely concur. Scharling's* *oxide of omichmyle* does not seem to me to differ in any of its properties from Proust's resin; but as Scharling, instead of evaporation, employed congelation as a means of concentrating the urine, and then extracted his so-called oxide with ether, there seems some reason for supposing that his substance may have preexisted in the urine. On examining the further details of his process, it will be found, however, that he used boiling caustic lye for the purpose of purifying it; and it need hardly be observed that no conclusion can be drawn regarding the preexistence of any organic compound which has passed through a process of purification involving the use of such an energetic agent as caustic alkali. In the course of his experiments on the constitution of urine, Liebig† also obtained the resinous substance of Proust, and he found it to possess in general the properties previously ascribed to it. The results of this portion of his investigation were summed up in the following words:—"From the preceding it follows that human urine contains, as organic acids, uric acid and hippuric acid, and another nitrogenous substance (most probably the colouring-matter of urine) which, in contact with air (it is only in contact with air that, as already observed by Gay-Lussac, the putrefaction of urine, accompanied by absorption of oxygen, takes place), is decomposed, yielding acetic acid and a resin-like substance."

In my paper on the occurrence of indigo-blue in urine, I gave a short account of some experiments on these brown colouring-matters, and the phenomena attending their formation. I there stated that "when muriatic or sulphuric acid is added to urine, the mixture on being heated becomes brown, and begins to deposit dark-brown flocks, which increase in quantity when the heating is continued. When these flocks are filtered off, washed, and dried, they form a compact dark-brown mass, from which cold alcohol extracts a resinous matter, leaving undissolved a brown powder, which dissolves, however, in a boiling mixture of alcohol and ammonia." These facts were previously known from the researches of Proust. I succeeded,

* *Annalen der Chem. und Pharm.*, B. xlii. S. 265.

† *Ibid.* B. i. S. 161.

however, in discovering two new facts, to which I attach some importance. The first is, that the composition of the brown pulverulent substance, which is little soluble in alcohol, stands in a definite relation to that of indigo-blue; the second, that the urine, after depositing these flocks and being made alkaline, has acquired the property of reducing oxide of copper, from which it may be inferred that it contains glucose in solution. As the analytical details which led to the discovery of the first fact have not hitherto been published, I think this a fitting occasion for making them known.

The brown pulverulent substance was prepared in the following manner:—Urine was mixed with hydrochloric acid and allowed to stand. The uric acid which was deposited was separated by filtration, and the liquid was boiled for some time. The black powder which separated during the boiling was filtered off, washed with water, dried, and treated with cold alcohol, which extracted the easily soluble resinous portion, thereby acquiring a brown colour. The portion left undissolved by the cold alcohol was dissolved in boiling alcohol to which a little ammonia was added. The brown solution was filtered and mixed with an excess of hydrochloric acid, which produced a brown precipitate, the supernatant liquid remaining coloured. This precipitate was collected on a filter, washed with cold alcohol until the acid and sal-ammoniac were removed, and dried. It had then the appearance of a dull, black, amorphous mass, which yielded a brownish-black powder, strongly resembling some of the products of decomposition of indican. When heated in a crucible it gave off a smell like that of burning horn, and then burned without previously fusing, giving much charcoal, which disappeared without leaving any ash. I need not describe its other properties, as they are in no way characteristic or interesting. Its composition, which is a matter of more importance, was determined by several analyses, the results of which are as follows:—

I. 0·4305 grm., dried at 100° C. and burnt with oxide of copper and oxygen, gave 0·9720 grm. carbonic acid and 0·1985 grm. water.

0·5815 grm., heated with soda-lime, gave 0·4190 grm. platinum.

II. 0·3850 grm., prepared on another occasion, gave 0·8760 grm. carbonic acid and 0·1755 grm. water.

0·5315 grm. gave 0·3685 grm. platinum.

These numbers lead to the formula $C_{14}H_7NO_4$, which requires—

	Calculation.		Experiment.	
			I.	II.
C_{14}	84	61·31	61·57	62·05
H_7	7	5·10	5·12	5·06
N	14	10·21	10·23	9·85
O	32	23·38	23·08	23·04
	137	100·00	100·00	100·00

Now the formula $C_{14}H_7NO_4$ is also that of anthranilic acid, the acid

formed by the action of alkalies and oxygen on indigo-blue; and though there is not the least resemblance between the two bodies, still the identity in composition seems to indicate the possibility of a common origin. Is it not possible, it may be asked, that the substance in urine which produces indigo-blue may be in part converted, by a process of oxidation, into some other substance which yields, instead of indigo-blue, a body having the composition of anthranilic acid, *i. e.* of a substance which is formed by the oxidation of indigo-blue? To me it seems very probable that this may be the case. I am, however, far from attaching great importance to the composition of this substance as just given, for on a subsequent occasion I obtained a product having exactly the same appearance as before, but a different composition. On this occasion the method of preparation was somewhat different. The urine was first mixed with acetate of lead as long as a precipitate was produced. To the filtered liquid there was added basic acetate of lead, which gave rise to a second precipitate. This was filtered off, washed with water, and treated with an excess of dilute sulphuric acid, and the filtered liquid, instead of being boiled, was poured into a shallow vessel and left to stand until, by spontaneous evaporation, it had become tolerably concentrated. On now adding cold water, a brown powder was left undissolved, which was filtered off, washed with boiling water, and then treated with boiling alcohol as long as anything was dissolved. The liquid, after being filtered boiling hot, was evaporated, and the residue was treated with a little cold alcohol, which left a brown powder undissolved. This was pressed between folds of blotting-paper and dried, after which it presented the same appearance as the first specimen. Its analysis led to the following results:—

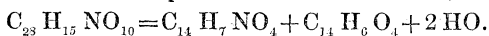
0.3730 grm. gave 0.8295 grm. carbonic acid and 0.1785 grm. water.

0.5750 grm. gave 0.2055 grm. platinum.

The formula $C_{23}H_{15}NO_{10}$, with which these numbers correspond, requires—

	Calculation.		Experiment.
C_{23}	168	60.64	60.65
H_{15}	15	5.41	5.31
N	14	5.05	5.07
O_{10}	80	28.90	28.97
	<hr/> 277	<hr/> 100.00	<hr/> 100.00

Now the two formulæ, though not identical, stand in a certain relation to one another. If to the first there be added the formulæ of benzoic acid and of water, the sum will represent the second formula, for



Benzoic acid is a product of decomposition of hippuric acid and other animal substances, and it need therefore cause no surprise to find its elements among organic groups occurring in animal secretions, though of course its actual presence in this case is doubtful.

These experiments render it probable that the ordinary brown colouring-matters formed by the action of acids on urine are in fact derivatives of indigo-blue, however little their properties may resemble those of the latter. In some experiments, an account of which I presented a short time ago to the Manchester Literary and Philosophical Society *, I obtained by the direct action of alcohol, acetate of soda, and caustic soda on indigo-blue a number of products, several of which bear a striking resemblance to *uromelanine*, as the brown pulverulent substance obtained by the action of acids on urine has been called, and which differ in their properties from indigo-blue quite as widely as that substance does. But into this part of the subject I cannot enter further at present.

The liquid filtered from the insoluble matter formed by the action of acids on urine I found to possess the property, after being made alkaline, of dissolving oxide of copper and converting it into suboxide on being boiled. This reaction, which had never been previously observed, I attributed to the presence of glucose, which, together with the brown colouring-matters, had been formed at the expense of the extractive matters. The correctness of this inference has been doubted, since the same reaction may be produced by other substances as well as glucose ; but whether it be correct or not, the fact remains, that normal urine free from sugar acquires the property of reducing oxide of copper as soon as it has been boiled with the addition of a strong acid. The general conclusion to which I was led by these few experiments was, that there not only exists a great resemblance between indican and the extractive matters of urine, as proved by the similarity of their products of decomposition, but that they are also very probably in some way closely related as regards their composition and general properties. In giving an account of my views on this subject I used these words :—"I think it is probable that the indigo-producing body will be found, as regards its formation and composition, to occupy a place between the substance of the tissues and the ordinary extractive matter of urine." Though this may have appeared at the time when it was pronounced a hasty conclusion, further research has only tended to confirm it.

III. The colouring-matters occurring in normal urine, and to which the usual colour of the secretion is due, have been less frequently submitted to investigation than those which make their appearance only exceptionally, or in consequence of some artificial process of decomposition. This circumstance may easily be accounted for. These substances are all amorphous and possess few characteristic properties ; hence their separation from the other constituents of urine is attended with great difficulties, and has even been pronounced impossible. They are also compounds of very little stability, as every one who has worked with them must have observed, so much so that mere evaporation of the urine seems to produce a complete change in their composition, as seen by the marked alteration of colour which takes place during the process. Then it has been observed that

* *Memoirs of the Society*, 3rd series, vol. iii. p. 66.

normal urine exhibits great diversity of tint without any corresponding difference in its other properties, and hence it has been inferred that these differences are of little physiological or pathological importance, and that an investigation of their cause would not be likely to lead to any useful practical results. Our knowledge of the properties and composition of these substances is therefore extremely defective, and the most discordant views prevail as to their true nature.

Foureroy and Vauquelin* were of opinion that the smell, colour, taste, and great liability to decomposition of urine, in fact all its characteristic properties, were due to one constituent only, viz. urea. It is evident, however, that their urea must have been impure, since they obtained from it by the action of caustic potash a brown fatty matter and acetic acid, products which could only have been derived from the extractive matter and other impurities with which it was contaminated. It was afterwards shown by Berzelius that urea is colourless, and possesses no remarkable smell nor taste. Proust, as mentioned above, attributed the colour, as well as the bitter taste and peculiar smell of urine, to his fallow resin. Prout thought that the colouring-matter of healthy urine was of two kinds, one of them being capable of combining with urate of ammonia and imparting to it the usual tint of uric acid calculi, the other destitute of this property. To Berzelius†, the great observer who has enriched almost every department of chemical science with his researches, we owe the first, it may almost be said the only, investigation of the extractive matters of urine, the substances to which, as he correctly supposed, the ordinary colour of the secretion is due. This investigation, though now almost forgotten, may still be consulted with advantage, as it contains information not to be found elsewhere. In its main results I have found it remarkably correct, and I shall have occasion to refer to it again. Though Berzelius did not succeed in obtaining his substances in a state of complete purity and free from other constituents of urine, such as urea and chlorides, he nevertheless ascertained the existence of several distinct urinary extractive matters, which were distinguished from one another by their behaviour towards various solvents. One of these he found to be soluble in absolute alcohol, the second was only soluble in alcohol of sp. gr. 0·833, while the third was insoluble in alcohol of all strengths, and only soluble in water. He seems also to have obtained a minute quantity of an extractive matter soluble in ether, the others being insoluble in that menstruum. The extractive matter soluble in absolute alcohol he proposed to name *halophile*, in consequence of its power of combining with various neutral salts. According to Berzelius, these substances bear a great resemblance to the extractive matters of flesh. Duvernoy made some experiments on these extractive matters, and he seems to have been the first to observe the remarkable deepening and change of colour which is seen on adding strong acids to their watery solutions. One of the methods employed by him for separating the colouring or extractive matter

* Annales de Chimie, t. xxxi. p. 68.

† Lehrbuch der Chemie, B. ix.

from the other constituents is worth mentioning. He added an excess of acid to urine, and the uric acid which separated on standing he treated with boiling alcohol, which left the acid undissolved, and, after filtration and evaporation, gave a residue consisting of a reddish-brown extractive matter, which had a bitter aromatic taste, and, when warmed, exhaled a urinous odour. The colour of its watery solution was exactly like that of urine.

In his elaborate memoir on urine, Lehmann * makes some remarks on the properties of the extractive matter of urine and the best method of preparing it. For the purpose of obtaining it in a state of purity, he submitted urine to congelation, and evaporated the concentrated liquid *in vacuo*, employing afterwards alcohol and ether for the purpose of extracting it from the residue. No part of the process described by him would induce any extensive decomposition of the substance under examination. On the other hand, it is very doubtful whether it was quite free from impurities, since he attributes to the coloured extractive matter (*färbender Extractivstoff*) of urine the property of inducing decomposition in urea, and consequently in urine also—a property which it certainly does not possess when pure, however liable it may itself be to decomposition. The putrefaction of urine, which manifests itself by the conversion of the urea into carbonate of ammonia, must be caused by some other body. The extractive matter does not act as a ferment, which may indeed be inferred from the very small quantity of nitrogen contained in it. The disagreeable odour which the watery solution of Lehmann's substance began to exhale when exposed to the air also points to some impurity. Its acid reaction he attributes to an admixture of lactic acid, which was generally supposed to be contained in urine, until its entire absence was proved by the experiments of Liebig. Lehmann's observations regarding its other properties, as, for instance, the changes of colour produced in its watery solution by various reagents, are, however, remarkably correct.

Lehmann, as well as Berzelius, found the substance to which healthy urine owes its colour to be completely soluble in water. Subsequently, however, most of the attempts which were made to isolate the colouring-matter of urine ended in the separation of substances quite insoluble in water. These must in all cases have been products of decomposition; for I consider it quite certain that all colouring-matters derived from urine which are insoluble in water are not contained as such in the secretion, provided the latter is in its normally acid state. In the experiments of Scherer † and Harley ‡, various products of decomposition of this kind seem to have been obtained. Scherer, not being satisfied with the methods of preparing and separating the extractive matters given by Berzelius, adopted one of his own, which yielded a brown humus-like substance, insoluble in water, but soluble in alcohol and alkalies. Scherer calls this

* Journal für praktische Chemie, B. xxv. S. 1.

† Annalen der Chemie und Pharmacie, B. lvii. S. 180.

‡ Pharmaceutical Journal, vol. xii. p. 243.

substance the colouring-matter of urine, though it must be evident to any one reading his account that it was a product formed by the action of hydrochloric acid on the extractive matter, and essentially the same as that previously obtained by Proust. Scherer submitted his substance to analysis, and found its composition to vary exceedingly. Hence it may be inferred that it consisted of a mixture of two or more substances. In my experiments on the brown colouring-matters formed by the action of acids, I obtained, as mentioned above, bodies having the same external appearance and general properties, but varying in composition. The latter corresponded on one occasion with the formula $C_{14}H_7NO_4$; on another occasion the analysis led to the formula $C_{28}H_{15}NO_{10}$. Now, on calculating the composition of a mixture of equal parts of the two bodies having respectively these formulæ, it will be found to agree tolerably well with the mean of the two first analyses given by Scherer, as will be seen on comparing his numbers with the calculated composition according to the formula

$$C_{42}H_{22}N_2O_{14} = C_{14}H_7NO_4 + C_{28}H_{15}NO_{10}.$$

	Calculation.	Scherer.
C	60·87	61·37
H	5·31	6·10
N	6·76	7·03
O	27·06	25·50
	<hr/> 100·00	<hr/> 100·00

It does not appear that Scherer took the precaution of treating his product with alcohol, in order to separate the easily soluble resinous matter which is always formed together with the pulverulent body when the extractive matters are decomposed by acids. Unless this precaution is taken, the product is sure to contain more than one substance, and its analysis must give very discordant results. On one occasion Scherer obtained by the direct action of hydrochloric acid on urine a dark-blue powder, which when dry assumed a coppery lustre like indigo, and must, indeed, have been indigo-blue itself. The formation of a blue colouring-matter by the action of acids on some constituent of urine had been observed by Heller only a short time previously. Neither of these chemists, however, was aware of its true character, which was not discovered until long afterwards.

Harley * succeeded in separating Scherer's colouring-matter into several substances, to only one of which, in his opinion, the colour of ordinary urine is to be attributed. This, according to his description, is a resinous, amorphous body of a fine red or brownish-red colour, insoluble in water, but easily soluble in alcohol, ether, and caustic alkalies, to which he gave the name of *urohæmatine*. On being incinerated it leaves a little oxide of iron, and hence Harley infers that it is allied to the hæmatine of blood, of which it is perhaps only a modification. By a process similar to that employed by Harley, Marcet † obtained from urine a resinous rose-coloured

* Journal für praktische Chemie, B. lxi. S. 264.

† Bibl. Univ. de Genève, 1852, p. 144.

substance, having an acid reaction, insoluble in water, but soluble in alcohol, and which he supposed must exist in the secretion in a free state. I have no doubt, however, that this and all similar bodies are products of decomposition derived from the extractive matters or the indigo-producing substance of the urine. They do not preexist in the secretion, but are formed during the process of preparation by the action of the reagents employed. Even a prolonged heating at 100° C. is quite sufficient, as I shall hereafter show, to produce a complete decomposition of the extractive matters, and their conversion into products of an entirely different nature, consisting in great part of brown resinous substances insoluble in water.

The investigations touching this subject which remain to be noticed are few in number. Notwithstanding its importance from a physiological point of view, the difficulties connected with it, and the uncertainty of the conclusions to which most previous researches had led, probably deterred many from entering on its investigation. Tichborne's* account of the normal urinary pigment differs from those of some of his predecessors. According to him the colouring-matter of normal urine is a brown, amorphous substance, which is very hygroscopic, easily soluble in water, less soluble in alcohol, and insoluble in ether. The colour of its watery solution cannot be distinguished from that of ordinary urine, and by making it more or less dilute, the several tints of normal urine may be imitated. Tichborne has given the results of its analysis, which is probably the first ever made of any urinary extractive matter, that is, of the substance as it exists in urine to which the usual colour of the secretion is due. His results, however, differ very widely from those arrived at by myself, and lead to a composition more nearly approaching that of the brown colouring-matters insoluble in water so often obtained in previous experiments. The hypothesis which he has ventured to set up, viz. that this substance is derived in some way from hippuric acid, is, I think, totally without foundation. Indeed there are more reasons in favour of the converse hypothesis, viz. that the urinary extractive matters are the source, or at least one source, of hippuric acid. The existence in urine of more than one kind of extractive matter seems to have escaped the notice of this observer.

By far the most complete investigation of the colouring-matters of normal urine is that of Dr. Thudichum†. The results of this investigation having quite recently been made known, I need not enter at present into any of the details. In giving an account of my own experiments, I shall have occasion to show that my results differ in many respects from those of Dr. Thudichum. I cannot, however, even now refrain from expressing my surprise that, notwithstanding the numerous observations and experiments of chemists on the blue and red colouring-matters from urine, he should have arrived at the conclusion that "from healthy human urine neither indican nor uroxanthine, nor any other substance yielding, by decomposition with

* *Chemical News*, vol. v. p. 171.

† *British Medical Journal*, November 5, 1864.

acids, indigo-red and indigo-blue, can be extracted," and that "it yields neither indigo-red nor indigo-blue by boiling with acids." This result, however, may easily be accounted for by any one conversant with the subject who attentively considers the details of his process. The indigo-producing body of urine, if it be not identical with indican, is certainly quite as susceptible of change as the latter; and the small quantity existing in the secretion may easily disappear under the influence of heat, alkalies, or fermentation, and become so changed as no longer to yield indigo-blue or indigo-red with acids. The nature of this change I have explained in my papers on the formation of indigo-blue. Now, Dr. Thudichum's process commences by adding to urine an excess of caustic baryta or lime. At subsequent stages he boils and evaporates his liquids with the assistance of heat. After operations such as he describes it is impossible that any trace of indican, or any body resembling it, can remain undecomposed. Unless certain precautions are adopted in conducting delicate experiments, only negative results can be expected.

The *alkaptone* of Bædeker*, and the colloid acid from urine lately described by Marcet†, probably stand in some relation to the ordinary extractive matters of urine, which they strongly resemble in most of their chemical and physical properties. The nature of the methods employed for the preparation of these bodies renders it, however, extremely doubtful whether they preexisted in the secretion, since in both cases solutions containing, together with the organic substances, strong mineral acids, were heated and even evaporated—a proceeding which must have led to the decomposition of the extractive matters, and the formation of bodies not previously existing.

The preceding account, in which I have endeavoured to present a summary of the results obtained in previous researches, will serve to give an idea of the present state of our knowledge on this subject; and I will now proceed to give an account of my own experiments. Before doing so, I may state that I shall apply the term "colouring-matter" to those bodies only which occur naturally in urine, or are formed by processes of decomposition, and which are insoluble, or not easily soluble, in water. The substances easily soluble in water, to which the colour of normal urine is due, I shall continue to call "extractive matters," until I shall have shown that they are bodies the properties and composition of which are sufficiently definite and unvarying to justify me in bestowing on them peculiar names.

The extractive matters being, as I believe, the source from which most of the colouring-matters of urine are derived, I resolved to commence the investigation by a careful examination of their properties and composition. The first step, indeed, which I thought it necessary to take before proceeding further at all was to ascertain whether these extractive matters are

* *Annalen der Chemie und Pharmacie*, B. cxvii. S. 98.

† *Proceedings of the Royal Society*, vol. xiv. p. 1.

bodies of a definite character, or whether they are merely accidental mixtures of various excrementitious substances thrown out by the system, and differing in their nature according to circumstances. In the former case a further exploration of this field would be justified by the probability of arriving at definite results. In the latter case, however, the investigation would certainly have to be abandoned at once, from the want of a secure basis on which to found further research.

In order to arrive at a positive conclusion on this point, the readiest means was, as it seemed to me, to ascertain the exact composition of the extractive matters obtained at different times from various sources; for, being neutral uncrystallizable bodies, it was evident that a mere examination of their chemical and physical properties would lead to no certain result. This portion of the investigation has occupied me for some time, commencing in the year 1856, and will form the subject of the present communication. The successive series of experiments which were made will be distinguished by letters with the respective dates attached.

A (1856).

In this, as well as in the subsequent series of experiments, I made use of neutral and basic acetate of lead for the purpose of separating the extractive matters from the other constituents of urine, the same means having previously been employed with this view by Scherer. Having taken a quantity of ordinary urine, I added to it a solution of acetate of lead, which produced a copious precipitate, consisting of sulphate, phosphate, chloride, and urate of lead, together with lead compounds of extractive matter. This precipitate was filtered off and thrown away. In the filtered liquid, which was lighter in colour than the original urine, basic acetate of lead produced a second precipitate as abundant as the first, and consisting principally of lead compounds of the extractive matters mixed with basic chloride of lead. This precipitate, after being well washed with water, was treated with an excess of cold dilute sulphuric acid, with which it was allowed to remain in contact for some time. The extractive matter set at liberty by the acid communicated to the liquid a brown colour, a peculiar urinous odour being at the same time evolved. The excess of acid was removed by adding carbonate of lead, and stirring the mixture well in a mortar. After all effervescence had ceased, the liquid, which was now of a fine yellow colour like urine itself, was filtered and evaporated; but in order to avoid any decomposition which might have been caused by the application of artificial heat, the evaporation was conducted at the ordinary temperature by means of a current of air in the apparatus formerly employed in the preparation of indican*. After evaporation there was left a thick brown syrup, which was poured into a flask and treated with cold alcohol, with which, after being well shaken, it was left to stand for some time. The alcohol left a portion of this syrup undissolved as a brown glutinous mass (*a*). The liquid, which had a deep yellow colour, was poured off, and there was added to it an alcoholic solution of acetate of lead, which produced a cream-

* See *Memoirs of the Manchester Literary and Philosophical Society*, vol. xiv. p. 183.

coloured precipitate. Having, as I supposed, added sufficient acetate of lead to precipitate about one half of the matter in solution, I filtered the liquid from the precipitate (*b*), and then added more of the lead solution, which produced a precipitate of a pure cream-colour (*c*). This was filtered off, washed with alcohol, dried *in vacuo*, and submitted to analysis. It contained, like many of the lead compounds subsequently analyzed, chloride of lead, which had to be estimated. By attempting to remove all the hydrochloric acid from the solution of extractive matter before precipitation, I ran the risk of producing decomposition in the organic substance.

I. 1·0895 grm. of this compound, burnt with oxide of copper and oxygen, gave 0·7765 grm. carbonic acid and 0·2175 grm. water.

1·1580 grm., burnt with soda-lime, gave 0·1355 grm. chloride of platinum and ammonium.

0·7780 grm. gave 0·6240 grm. sulphate of lead.

0·9190 grm. dissolved in nitric acid gave with nitrate of silver 0·0795 grm. chloride of silver, equivalent to 0·0772 grm. chloride of lead.

These numbers lead to the following composition :—

C	19·43
H	2·21
N	0·73
O	16·97
PbO	52·26
PbCl	8·40
	<hr/> 100·00

After deducting the oxide and chloride of lead, and calculating the composition in 100 parts of the organic substance combined with them, this composition will be found to correspond with the formula $C_{62}H_{39}NO_{40}$, which requires

	Calculation.		Experiment.
C_{62}	372	49·93	49·39
H_{39}	39	5·23	5·61
N	14	1·87	1·85
O_{40}	320	42·97	43·15
	<hr/> 745	<hr/> 100·00	<hr/> 100·00

No importance is to be attached to this formula, which is merely an empirical expression for the composition of the substance, or mixture of substances, prepared in the manner described. As a guide to further experiments, the analysis was, however, not without use. The very small proportion of nitrogen obtained showed that the substance was probably free from the urinary constituents containing much of that element, and that the lead compound contained extractive matter only; but whether the latter consisted of only one substance or of several was doubtful, as the method of preparation afforded no guarantee for its purity.

The lead precipitate (*b*), which, it was to be presumed, had the same composition as the one analyzed, was now examined. It was suspended in

water and decomposed with sulphuretted hydrogen, and the filtered liquid was evaporated by means of a current of air in the apparatus above referred to. During evaporation some white crystals were deposited, consisting probably of a product of decomposition formed by the action of the free hydrochloric acid on the extractive matter. These were filtered off, and the liquor was evaporated as before to a syrup, which was treated with cold alcohol. The alcoholic solution, after being filtered from some white crystals which were left undissolved, was evaporated to a syrup, which, after being mixed with a little alcohol for the sake of dilution, was poured into a flask and agitated with successive doses of ether as long as anything was taken up by the latter. The residue left undissolved by the ether (*d*), after the ethereal liquid (*c*) had been poured off, was treated with cold alcohol, which dissolved the whole with the exception of some white crystals*. The filtered liquid was mixed with acetic acid and then with acetate-of-lead solution, and the brown precipitate caused by the latter having been filtered off, there was added to it a small quantity of ammonia, which produced a cream-coloured precipitate. This precipitate was filtered off, washed with alcohol, dried *in vacuo*, and employed for the following analysis:—

II. 1·3735 grm. gave 0·7420 grm. carbonic acid and 0·2345 grm. water.

1·5800 grm. gave 0·1395 grm. chloride of platinum and ammonium.

0·7210 grm. gave 0·6570 grm. sulphate of lead.

1·8070 grm. gave 0·0755 grm. chloride of silver, equivalent to 0·0733 grm. chloride of lead.

These numbers lead to the following composition:—

C.....	14·73
H.....	1·89
N.....	0·55
O.....	14·99
PbO	63·78
PbCl	4·06
	<hr/> 1·0000

* These crystals consisted probably of the same substance as those deposited during the evaporation of the watery solution filtered from the sulphide of lead. They contained, besides organic matter, a quantity of sulphates of earthy bases. The latter were removed by dissolving the whole in water and adding an excess of caustic baryta. After passing carbonic acid through the filtered liquid, evaporating to dryness, treating the residue with boiling water, filtering, and again evaporating to dryness, a white crystalline mass was obtained, which was free from all inorganic impurities. A few of the properties of this substance may be mentioned, though they are not sufficient to identify it. When heated on platinum-foil it melted and then burned, leaving much charcoal, which, however, disappeared entirely on being further heated. On being heated in a tube, it gave a little crystalline sublimate. It dissolved with difficulty in boiling alcohol, and the solution, on cooling, deposited some transparent, prismatic crystals. The watery solution remained unchanged on the addition of acetate of lead, but on adding ammonia there was an abundant white precipitate. On mixing the watery solution with a salt of copper and an excess of caustic soda it turned blue, but remained unchanged on being boiled.

The simplest formula with which the composition of the substance, combined with the oxide and chloride of lead, agrees is $C_{62}H_{46}NO_{47}$, which requires

	Calculation.		Experiment.
C_{62}	372	46·03	45·80
H_{46}	46	5·69	5·87
N	14	1·73	1·71
O_{47}	376	46·55	46·62
	808	100·00	100·00

This analysis, though, like the first, of little value in itself, seems to point to the conclusion that the extractive matter has a tendency to undergo a change, which consists in the absorption of water, and which is probably promoted by the action of strong acids. There is, however, another way of explaining its results, which will be given when I come to review the whole of the analytical data.

A portion of the alcoholic solution from which this lead compound was precipitated was evaporated, when it left a brown syrup, some of the properties of which are not without interest. When heated in a crucible it began to boil, evolved acid fumes, consisting partly of hydrochloric acid, and left after combustion much charcoal, which burnt away, leaving a little white ash. The watery solution was strongly acid. After being mixed with a solution of oxide of copper and an excess of caustic alkali it became green, and, on being boiled, the liquid deposited suboxide of copper; but this reaction was probably due to an admixture of some impurity or of some product of decomposition. On adding to the alcoholic solution an alcoholic solution of acetate of lead, a cream-coloured precipitate fell, which, after being filtered off and washed with alcohol, was treated with dilute sulphuric acid. The filtered liquid, after being made alkaline, did not reduce oxide of copper; but, on the other hand, the liquid filtered from the cream-coloured precipitate gave with ammonia a white precipitate, which, on being treated in the same way as the other, was found to contain, in combination with oxide of lead, a substance which, in conjunction with caustic alkali, readily reduced the oxide. The lead compound, the analysis of which has just been given, may indeed, as I shall show further on, have consisted of a mixture of equal parts of two lead compounds, viz. of the compound of an extractive matter and of that of another body having the composition of glucose. The watery solution of the syrup, on being mixed with hydrochloric acid and boiled, became brown, and deposited dark brown flocks. The filtered liquid left on evaporation a residue, which, on being treated with water, dissolved only in part, an additional quantity of brown flocks being left undissolved. These flocks were very little soluble in boiling alcohol, but they dissolved readily in a mixture of alcohol and ammonia. The liquid filtered from the flocks left on evaporation a yellow syrup mixed with a quantity of needle-shaped crystals arranged in star-shaped masses.

On being dried, the syrup became hard, but deliquesced again on exposure to the air.

The ethereal liquid (e) containing in solution that portion of the extractive matter of the lead precipitate (b) soluble in ether, was agitated with carbonate of lead, by which means the hydrochloric acid contained in it was entirely removed. To the filtered liquid there was added an alcoholic solution of acetate of lead, which produced a cream-coloured precipitate. This was filtered off, washed with cold alcohol, dried *in vacuo*, and analyzed, the results obtained being as follows:—

III. 1·3455 grm. gave 1·0425 grm. carbonic acid and 0·2730 grm. water.

1·5580 grm. gave 0·1385 grm. chloride of platinum and ammonium.

0·7565 grm. gave 0·6025 grm. sulphate of lead.

The compound contained therefore, in 100 parts,

C	21·13
H	2·25
N	0·55
O	17·47
PbO	58·60
	<hr/> 100·00

The composition of the substance, combined in this case with oxide of lead, agrees tolerably well with the formula $C_{86}H_{51}NO_{52}$, which requires

	Calculation.		Experiment.
C_{86}	516	51·75	51·04
H_{51}	51	5·11	5·43
N	14	1·40	1·34
O_{52}	416	41·74	42·19
	<hr/> 997	<hr/> 100·00	<hr/> 100·00

In the case of a compound like this, having such a high atomic weight, several formulæ may of course be calculated, each of which may give a theoretical composition agreeing as well as the above with that found by experiment. My reasons for adopting the one just given will be stated further on.

A portion of the liquid from which this compound was precipitated with acetate of lead was evaporated, when it left a brown syrup closely resembling that obtained from the liquid from which the lead compound of the second analysis was precipitated. When heated in a crucible it gave off copious fumes, and left off much charcoal, which, however, burnt away, leaving only a trace of ash. Its watery solution had a strongly acid reaction, though it was quite free from hydrochloric acid. When treated with boiling caustic soda lye it evolved ammonia. The watery solution, on the addition of a salt of copper and an excess of caustic alkali, became green, and the filtered liquid, on being boiled, deposited an abundance of

suboxide of copper. This reaction was, however, due to some substance accompanying the extractive matter. On adding to the alcoholic solution of the syrup an alcoholic solution of acetate of lead, a cream-coloured precipitate was produced which contained none of this substance; but on adding ammonia to the filtered liquid, a white precipitate fell in which it was contained in combination with oxide of lead. The lead compound analyzed was therefore free from this impurity, and probably contained merely the urinary extractive matter soluble in alcohol and ether. The watery solution of the syrup, on being mixed with hydrochloric acid and boiled, became darker in colour, and then deposited dark brown resin-like masses, which remained in a state of fusion as long as the liquid was kept boiling. The filtered liquid left on evaporation a syrupy residue, which was only partly soluble in water, a quantity of the resin-like substance being left undissolved. The solution, after being again filtered and evaporated, left a brown syrup filled with crystalline needles. Cold alcohol dissolved the greatest part of this residue, leaving only the crystalline needles undissolved. The resinous substance, after being well washed with water, was treated with cold alcohol, in which it was entirely soluble, forming a brown solution which, on evaporation, left a brown, shining, brittle residue. The extractive matter contained in this compound differs therefore from that of the lead compound previously analyzed, not only by its solubility in ether, but also by its yielding with acids products of decomposition of a different kind.

The alcoholic liquid filtered from the lead precipitate (*b*) was mixed with more acetate of lead and some ammonia, with which it gave a bulky cream-coloured precipitate. This was filtered off, washed with water, then suspended in water, and decomposed with sulphuretted hydrogen. The filtered liquid was evaporated, as before described, by means of a current of air, and the yellow syrup left on evaporation was poured into a flask and agitated with alcohol, which dissolved the whole of it with the exception of a slight residue, consisting of a glutinous substance mixed with some white crystals. The filtered liquid was evaporated, and the residue left was agitated with ether. The ether having been poured off, the insoluble portion was treated with cold alcohol, which dissolved almost the whole of it. The solution was mixed with a little alcoholic solution of acetate of lead, and the precipitate thereby produced having been filtered off, more acetate of lead was added, which gave a precipitate of a pure cream-colour. This was filtered off, washed with alcohol, dried *in vacuo*, and analyzed, the following results being obtained:—

IV. 1.0155 grm. gave 0.6730 grm. carbonic acid and 0.2055 grm. water.

1.1440 grm. gave 0.1425 grm. chloride of platinum and ammonium.

0.4165 grm. gave 0.3180 grm. sulphate of lead.

0.8905 grm. gave 0.0290 grm. chloride of silver, equivalent to 0.0282 chloride of lead.

In 100 parts it contained therefore

C.....	18·07
H.....	2·24
N.....	0·78
O.....	22·12
PbO.....	53·63
PbCl	3·16
	<hr/> 100·00

Though the substance combined in this case with the oxide and chloride of lead was without doubt a mixture, still I think it may be of use to devise some formula which shall express its composition, and thus lead to some plausible conjecture as to its constituents. The relatively large quantity of oxygen contained in it makes this rather difficult; but, as I shall presently show, one of the urinary extractive matters is richer in oxygen than the others, and by assuming that the substance in this case was a mixture of equal parts of this extractive matter and glucose, I arrive at the formula $C_{50}H_{39}NO_{44}$, which requires

	Calculation.		Experiment.
C_{50}	300	42·55	41·81
H_{39}	39	5·53	5·18
N.....	14	1·98	1·80
O_{44}	352	49·94	51·21
	<hr/> 705	<hr/> 100·00	<hr/> 100·00

The liquid filtered from this lead compound did in fact contain a substance having the composition of glucose. On adding to it an excess of ammonia, a bulky precipitate fell, which was filtered off and treated with a mixture of alcohol and acetic acid, in which almost the whole dissolved. To the filtered liquid there was added a small quantity of ammonia, which produced an almost white precipitate. This was filtered off, washed with alcohol, dried *in vacuo*, and analyzed.

1·3730 grm. of this precipitate gave 0·6585 grm. carbonic acid and 0·2265 grm. water.

2·0730 grms. gave 0·0600 grm. chloride of platinum and ammonium.

0·7945 grm. gave 0·7720 grm. sulphate of lead.

In 100 parts the compound contained therefore

C.....	13·08
H	1·83
N.....	0·18
O.....	13·42
PbO	71·49
	<hr/> 100·00

If the small percentage of nitrogen, which was probably due to an admixture of extractive matter be neglected, this composition leads to the formula $C_{24}H_{19}O_{10} + 7PbO$, which requires

C ₂₄	144	13·10
H ₁₀	19	1·72
O ₁₆	152	13·85
7 PbO	784	71·33
	<hr/> 1099	<hr/> 100·00

The brown glutinous mass (*a*) which was left undissolved by cold alcohol was treated with cold water, and the liquid, after being filtered from the insoluble matter (*f*), which consisted for the most part of chloride of lead, was evaporated as usual by means of a current of air. During evaporation some more chloride of lead was deposited, which was separated, and there was left at last a thin syrup, which was poured into a flask and agitated with alcohol. The alcohol converted it into a milky emulsion, which, after standing some time, deposited a brown glutinous substance, the supernatant liquid becoming clear. The latter having been poured off, the deposit was dissolved in cold water, and to the solution there was added acetate of lead and sufficient ammonia to cause a slightly alkaline reaction. The precipitate (*g*) which was thereby produced was filtered off, washed with water, and, after being suspended in water, decomposed with sulphuretted hydrogen. The liquid filtered from the sulphide of lead was evaporated in the air-current, when it left a brown syrup, which was treated with alcohol. The alcoholic liquid, after being filtered from some insoluble matter, consisting of crystals mixed with a little glutinous substance, was evaporated as before to a syrup, and this syrup was poured into a flask and agitated with successive portions of ether until nothing more was dissolved. The ethereal solution (*h*) having been poured off, the insoluble matter was treated with cold alcohol, which dissolved almost the whole of it. To the filtered liquid there was added a little alcoholic solution of acetate of lead; and the precipitate thereby produced having been filtered off, more lead solution was added, which gave a copious precipitate (*i*) of a greyish cream-colour. This, after being filtered off and treated in the same manner as the other lead precipitates, was analyzed, the results obtained being as follows:—

V. 1·3455 grm. gave 0·8455 grm. carbonic acid and 0·2830 grm. water.

1·5710 grm. gave 0·2490 grm. chloride of platinum and ammonium.

0·8830 grm. gave 0·7065 grm. sulphate of lead.

1·4975 grm. gave 0·2580 grm. chloride of silver, equivalent to 0·2508 grm. chloride of lead.

In 100 parts it was therefore composed as follows:—

C.....	17·13
H	2·33
N.....	0·99
O.....	17·38
PbO	45·43
PbCl	16·74
	<hr/> 100·00

If the formula $C_{38}H_{23}NO_{29}$ be adopted as expressing the composition of the organic substance combined with the oxide of lead, the relation in which the latter stands to the other extractive matters will be easily seen, though the calculated composition does not in this case agree very well in all respects with that deduced from the above analysis. This formula requires

	Calculation.		Experiment.
C_{38}	228	45·41	45·30
H_{23}	28	5·57	6·17
N	14	2·78	2·63
O_{29}	232	46·24	45·90
	502	100·00	100·00

It may excite some surprise that the extractive matter contained in this compound, which was soluble in alcohol, should have been obtained from the brown glutinous mass (*a*), which was insoluble in alcohol, and had quite the appearance of an extractive matter itself. This may, however, be easily explained, since the mass (*a*) contained lead, being, indeed, merely a lead compound of the extractive matter soluble in alcohol, and many of the compounds of the latter with bases are insoluble in alcohol. It is, in fact, still doubtful whether urine does contain an extractive matter insoluble in alcohol, and whether the various substances having this character, obtained in these and previous experiments, are not compounds of extractive matters with bases; but to this point I shall return on a future occasion.

The ethereal solution (*b*) contained some extractive matter, and also hydrochloric acid. The latter having been removed by introducing carbonate of lead and shaking well, there was added after filtration an excess of an alcoholic solution of acetate of lead, which produced a copious precipitate. This was filtered off and then dissolved in a mixture of alcohol and acetic acid. To the filtered solution a small quantity of ammonia was added. The precipitate thereby produced was filtered off and treated as usual before being analyzed.

VI. 1·2650 grm. of this compound gave 0·6860 grm. carbonic acid and 0·2025 grm. water.

1·4445 grm. gave 0·6810 grm. chloride of platinum and ammonium.

0·4610 grm. gave 0·4250 grm. sulphate of lead.

In 100 parts it contained therefore

C.....	14·79
H	1·77
N	0·35
O.....	15·26
PbO	67·83
	100·00

The substance combined with oxide of lead contained, in 100 parts,

C	45·97
H	5·52
N	1·08
O	47·43
	<hr/> 100·00

It will be seen that this analysis yielded results not differing very widely from those of the second analysis. The composition of the substance may therefore be explained in the same manner.

To the liquid filtered from the lead precipitate (*g*) there was added an excess of ammonia, which produced a pale cream-coloured precipitate. This was filtered off, washed with water, then suspended in water, and decomposed with sulphuretted hydrogen. The filtered liquid was poured into a flask, and there was added to it a quantity of freshly precipitated oxide of mercury, together with some metallic mercury, the whole being frequently shaken. By this means the hydrochloric acid contained in it was completely removed, after which the liquid was filtered and evaporated. The residue was treated with water, which left a little yellowish powder undissolved. Through the filtered liquid sulphuretted hydrogen was passed in order to precipitate the mercury in solution, and after being again filtered it was evaporated, when it left a pale yellow syrup. This was treated with alcohol, which dissolved almost the whole of it. To the filtered alcoholic solution there was added a solution of acetate of lead, which produced an almost white precipitate. This was filtered off and prepared as usual for analysis.

VII. 0·8440 grm. of this compound gave 0·6255 grm. carbonic acid and 0·2110 grm. water.

1·1035 grm. gave 0·0950 grm. chloride of platinum and ammonium.

0·4535 grm. gave 0·3155 grm. sulphate of lead.

In 100 parts it contained therefore

C	20·21
H	2·77
N	0·54
O	25·29
PbO	51·19
	<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C	41·41
H	5·69
N	1·10
O	51·80
	<hr/> 100·00

In this, as in several previous cases, the compound contained more than

one substance in combination with oxide of lead. Several other compounds similar to this were subsequently analyzed, and it will therefore conduce to clearness to pass them all under review together.

The insoluble matter (*f*), consisting chiefly of chloride of lead, still remained to be examined. After being washed with water, it was treated with cold dilute sulphuric acid. The liquid filtered from the sulphate of lead was mixed with an excess of baryta-water, which produced a brown precipitate consisting of sulphate of baryta mixed with some compound of extractive matter. To the filtered liquid there was added a slight excess of acetic acid, and then acetate of lead and sufficient ammonia to neutralize the acid. This gave a pale yellow precipitate (*i*), which was filtered off, washed, and decomposed with sulphuretted hydrogen. The resulting solution was filtered and evaporated. The residue left on evaporation was treated with alcohol, which left a little white matter undissolved. To the filtered liquid there was added an alcoholic solution of acetate of lead, which gave a yellowish precipitate. This was filtered off, washed with alcohol, and treated as usual previous to its being analyzed.

VIII. 1·2980 grm. of this compound gave 0·9555 grm. carbonic acid and 0·2520 grm. water.

1·5655 grm. gave 0·2575 grm. chloride of platinum and ammonium.

0·7115 grm. gave 0·5320 grm. sulphate of lead.

1·2690 grm. gave 0·1350 grm. chloride of silver, equivalent to 0·1312 grm. chloride of lead.

In 100 parts it contained therefore

C	20·07
H.....	2·15
N.....	1·03
O	19·70
PbO.....	46·71
PbCl	10·34
	<hr/> 100·00

The substance combined with the oxide and chloride of lead contained, in 100 parts,

C.....	46·74
H.....	5·02
N.....	2·40
O.....	45·84
	<hr/> 100·00

To the liquid filtered from the lead precipitate (*i*), which was employed for the preparation of the last compound, there was added more acetate of lead and an excess of ammonia. The bulky precipitate thereby produced was filtered off, completely washed with water, and then decomposed with sulphuretted hydrogen. The filtered liquid, which had the colour of urine, was mixed with oxide of mercury and left to stand for some time. It was then filtered again and agitated with metallic mercury, by which means the

chloride of mercury contained in it was converted into subchloride, and the liquid was rendered free from chlorine. The filtered liquid, which was almost colourless, was evaporated in the usual manner by means of an air-current, until its volume was considerably diminished. During evaporation a little white matter was deposited which was filtered off. The mercury contained in the solution was removed by means of sulphuretted hydrogen, and after being filtered it was evaporated as before to a syrup. This syrup was found to be insoluble in cold alcohol. It was therefore dissolved in a little water. To the solution there was added a little acetate of lead, which gave a slight precipitate, and this having been filtered off, the liquid was mixed with alcohol until no more precipitate was produced. This precipitate, which was white, was filtered off, washed with alcohol, and dried *in vacuo*. On being analyzed it yielded the following results:—

IX. 1·2825 grm. gave 0·6845 grm. carbonic acid and 0·2145 grm. water.

1·5205 grm. gave 0·0705 grm. chloride of platinum and ammonium.

0·7470 grm. gave 0·6690 grm. sulphate of lead.

In 100 parts it contained therefore

C.....	14·53
H.....	1·85
N.....	0·29
O.....	17·42
PbO.....	65·89
	<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C.....	42·67
H.....	5·44
N.....	0·85
O.....	51·04
	<hr/> 100·00

The two last analyses will suffice to show that the chloride of lead deposited during the evaporation of the liquid obtained from the precipitate, which basic acetate of lead produces in urine after the addition of neutral acetate, takes down with it a portion of the extractive matter, and that the composition of this portion is similar to that of the rest which is retained in solution.

This series of experiments leads to the conclusion that the precipitate produced in urine by basic acetate of lead contains, in combination with oxide and chloride of lead, at least two extractive matters, one of which is soluble in alcohol and ether, the other soluble in alcohol only, but very similar to one another in all other respects; and that it also affords a substance which has the composition and some of the properties of glucose, this being probably a product derived from one or both of the extractive matters, and not preexisting in the urine or even in the lead precipitate itself.

B (1857).

Having by the previous experiments determined in a general way the composition of the precipitate produced in urine by basic acetate of lead, I now resolved to ascertain whether the precipitate with neutral acetate of lead contains any urinary extractive matter in addition to the sulphate, phosphate, chloride, and urate of lead, of which it chiefly consists. For this purpose acetate of lead was added to urine, and the precipitate thereby produced was washed with water and then treated with an excess of dilute sulphuric acid, with which it was left to stand for some time. To the filtered liquid, which had a deep yellow colour, there was added sufficient baryta-water to remove the sulphuric acid, and then an excess of milk of lime, which gave a gelatinous precipitate consisting chiefly of phosphate of lime. The liquid, which had now lost much of its colour, was filtered and made acid with acetic acid. Acetate of lead now produced no precipitate but on the addition of ammonia a cream-coloured precipitate fell, which after being washed was treated with dilute sulphuric acid. The excess of acid was removed by means of carbonate of lead, and the filtered liquid was evaporated in the usual manner in a current of air to a syrup. This syrup was dissolved in cold alcohol, and to the solution there was added an alcoholic solution of acetate of lead. The precipitate thereby produced was filtered off, washed with alcohol, and then treated with dilute sulphuric acid. The excess of the latter having been removed as before by means of carbonate of lead, the filtered liquid was evaporated. The residue which was left was treated with cold alcohol, and the alcoholic solution, after being filtered, was mixed with twice its volume of ether, which caused it to become milky. After some time a glutinous deposit settled at the bottom of the vessel, leaving a supernatant liquid, which was bright yellow and clear. To this liquid there was added an alcoholic solution of acetate of lead, which produced a cream-coloured precipitate. This was filtered off, washed with alcohol, dried *in vacuo*, and analyzed as usual, the results being as follows:—

- I. 0·9805 grm. gave 0·8425 grm. carbonic acid and 0·2320 grm. water.
 1·1795 grm. gave 0·1850 grm. chloride of platinum and ammonium.
 0·6775 grm. gave 0·5000 grm. sulphate of lead.
 0·7280 grm. gave 0·0120 grm. chloride of silver, equivalent to 0·0166 grm. chloride of lead.

Hence the compound contained, in 100 parts,

C.....	23·43
H.....	2·62
N.....	0·98
O.....	18·34
PbO.....	53·03
PbCl	1·60
	<hr/> 100·00

The composition of the substance, combined in this case with oxide and chloride of lead, corresponds, if the great excess of hydrogen be disregarded, with the formula $C_{62}H_{36}NO_{37}$, which requires

	Calculation.		Experiment.
C_{62}	372	51·81	51·66
H_{36}	36	5·01	5·79
N	14	1·95	2·17
O_{37}	296	41·23	40·38
	718	100·00	100·00

This formula differs, as will be seen, only by three atoms of water from that to which the first analysis of the preceding series led, though, like the latter, it does not represent a pure unmixed substance. It may therefore be inferred that the precipitate produced in urine by neutral acetate of lead contains the same extractive matters as the precipitate which basic acetate of lead gives in the filtrate. This conclusion was confirmed in a very satisfactory manner by subsequent experiments.

C (1857).

The facility with which the hydrochloric acid derived from the chloride of sodium of the urine is removed from solutions of urinary extractive matters containing the acid, by means of oxide of mercury and metallic mercury, led me to try this method of purification on a somewhat larger scale than before. For this purpose a quantity of urine was mixed with acetate of lead, and to the liquid filtered from the precipitate basic acetate of lead was added, which produced, as usual, a second precipitate. This precipitate was filtered off, washed, and treated with dilute sulphuric acid. The excess of acid having been removed by means of carbonate of lead, the filtered liquid was evaporated in the air-current. The residue left on evaporation was treated with cold water, which left undissolved a mixture or compound of chloride of lead and extractive matter. This was filtered off, and the lead still contained in solution was precipitated by a current of sulphuretted hydrogen. The filtered liquid containing hydrochloric acid was now agitated with freshly precipitated oxide of mercury, to which some metallic mercury was added. As soon as it had become free from chlorine, it was again filtered and evaporated in the air-current. The glutinous residue left on evaporation was treated with cold alcohol, in which only a trace dissolved. That which was left undissolved by the alcohol was now dissolved in water, and through the solution, which contained an abundance of mercury, a current of sulphuretted hydrogen was passed, and the filtered liquid was evaporated. The residue was treated with cold alcohol. A pale yellow glutinous substance was left undissolved, which was dissolved in water. The watery solution was mixed with ammonia, with which it gave a flocculent precipitate. To the filtered liquid there was added acetate of lead, and the precipitate thereby produced was filtered off, washed, and

dissolved in acetic acid. The filtered solution was mixed with a large quantity of alcohol, which produced a pale cream-coloured precipitate. This was filtered off and prepared for analysis in the usual manner.

I. 1.3755 grm. of this compound gave 0.8125 grm. carbonic acid and 0.2770 grm. water.

2.0605 grms. gave 0.1175 grm. chloride of platinum and ammonium.

0.6830 grm. gave 0.5785 grm. sulphate of lead.

These numbers lead to the following composition :—

C	16.11
H	2.23
N	0.35
O	18.99
PbO	62.32
	<hr/> 100.00

The composition of the organic substance, combined with the oxide of lead, may be expressed by the formula $C_{86}H_{71}NO_{76}$, which requires

	Calculation.		Experiment.
C_{86}	516	42.68	42.75
H_{71}	71	5.87	5.93
N	14	1.15	0.95
O_{76}	608	50.30	50.37
	<hr/> 1209	<hr/> 100.00	<hr/> 100.00

It will be seen that the Analyses VII. and IX. of Series A, which were made with compounds prepared in the same manner as this, led to almost the same composition. It must not be inferred from this that these were all compounds of a pure substance with oxide of lead. It is more probable that the oxide of mercury employed in their preparation had simply the effect of removing certain substances from the solution with which it was brought into contact, leaving a mixture of others, the quantities of which stood in a certain unvarying ratio to one another. That the above formula represents a mixture containing glucose is very probable, as I shall afterwards show; and, on the other hand, it is certain that a great part of the extractive matters entered into combination with the oxide of mercury, as the solutions, after being shaken up with the oxide, became several shades lighter in colour. It is also not improbable that oxide of mercury causes the extractive matters to undergo a certain degree of oxidation—a conclusion to which the large percentage of oxygen yielded by the analysis just given, as well as the two others, seems to point. In my subsequent experiments I therefore ceased to employ oxide of mercury except as a means of purifying the extractive matter insoluble in ether and alcohol.

The mixture or compound of chloride of lead and extractive matter obtained as usual in this experiment was employed for the preparation of a compound containing less chloride of lead, the process being the same as

that adopted in preparing for Analysis VIII. of Series A. It was also analyzed, but the results present nothing of interest.

D (1857).

The lead compounds next analyzed were procured from the same source as those of Series A. Urine was mixed with a solution of acetate of lead as long as a precipitate was produced, and to the filtered liquid basic acetate of lead was added, which gave, as usual, an abundant precipitate. This was allowed to settle, washed, filtered off, and then treated with cold dilute sulphuric acid. The excess of the latter having been removed by means of carbonate of lead, the filtered liquid was evaporated in the usual manner by means of a current of air. The syrup which was left behind was dissolved in a little water, and the solution was mixed with a large quantity of alcohol. To the filtered liquid there was added an alcoholic solution of acetate of lead and some ammonia. The precipitate thereby produced was filtered off, washed with alcohol, and then treated with dilute sulphuric acid. The excess of acid having been removed with carbonate of lead, the filtered liquid was evaporated in the usual manner to a syrup. This syrup was poured into a flask together with a little alcohol, and a large quantity of ether was then added, which caused the separation of a glutinous substance that was slowly deposited at the bottom of the vessel. After the ethereal solution had become clear, it was poured off from the glutinous deposit (*a*) and evaporated, and the residue was treated with water, which left a little fatty matter undissolved. The filtered liquid was evaporated in the usual manner to a syrup, which was dissolved in alcohol. On the addition of acetate of lead and a little ammonia to this solution, a cream-coloured precipitate fell, which was filtered off, washed with alcohol, dried *in vacuo*, and analyzed. It contained no trace of chloride of lead.

I. 0.9905 grm. gave 0.7145 grm. carbonic acid and 0.2030 grm. water.

1.2625 grm. gave 0.1285 grm. chloride of platinum and ammonium.

0.6400 grm. gave 0.5265 grm. sulphate of lead.

These numbers correspond, in 100 parts, to

C	19.67
H	2.27
N	0.63
O	16.90
PbO	60.53
		<hr/> 100.00

The substance combined with oxide of lead contained, in 100 parts,

C	49.83
H	5.75
N	1.62
O	42.80
		<hr/> 100.00

It will be seen that the composition of the substance combined with oxide

of lead is nearly the same as that to which Analysis I. Series A. led, and which was expressed by the formula $C_{62}H_{39}NO_{40}$. It may also be remarked as a singular circumstance, that the quantity of oxide of lead found in the present analysis is equal to the sum of the oxide of lead and chloride of lead ($52.26 + 8.40$) of the former. Hence it may be inferred that in these compounds the chloride of lead and oxide of lead replace one another weight for weight, and not according to their respective equivalents. The same circumstance occurred on several other occasions*, otherwise it might have been attributed to mere accident.

The glutinous substance (*a*), insoluble in the mixture of alcohol and ether, was treated with water. The liquid, after being filtered from some undissolved matter, consisting chiefly of chloride of lead, was mixed with a solution of acetate of lead and a large quantity of alcohol, which produced a cream-coloured precipitate. This was filtered off, and after being treated in the usual manner, submitted to analysis, the results being as follows:—

II. 1.5515 grm. gave 0.9650 grm. carbonic acid and 0.3295 grm. water.

2.0575 grms. gave 0.3430 grm. chloride of platinum and ammonium.

0.9105 grm. gave 0.6875 grm. sulphate of lead.

1.1730 grm. gave 0.2340 grm. chloride of silver, equivalent to 0.2274 grm. chloride of lead.

These numbers lead to the following composition:—

C	16.96
H	2.35
N	1.04
O	20.24
PbO	40.02
PbCl	19.39
		<hr/> 100.00

The composition of the organic portion of the compound may in this case be represented by the formula $C_{38}H_{29}NO_{34}$, which requires

	Calculation.		Experiment.
C_{38}	228	41.99	41.79
H_{29}	29	5.34	5.81
N	14	2.57	2.57
O_{34}	272	50.10	49.83
	<hr/> 543	<hr/> 100.00	<hr/> 100.00

The mixture of extractive matter and chloride of lead obtained in this case was treated in the manner before described, and a compound was obtained which was also analyzed; but the details need not be given as

* For example, the percentage of oxide of lead of the sixth analysis of Series A (67.83) is equal to the sum of the oxide of lead and chloride of lead of the second analysis of the same series (67.84), the amount of other constituents yielded by the two analyses being nearly the same.

they possess no interest. I may state, however, that it contained 40 per cent. of oxide of lead and 35 per cent. of chloride of lead, and that the composition of the remaining 25 per cent. of organic substance did not differ very widely from that to which the preceding analysis conducted.

E (1858).

After having added to urine an excess, first of acetate and then of basic acetate of lead, the liquid is found to have lost the greatest part of its colour. Nevertheless it produces with ammonia a bulky precipitate, which is similar in appearance to the two other lead precipitates, and also contains some extractive matter. The object of this series of experiments was to ascertain whether the composition of this portion of extractive matter is the same as that of the portion contained in the two other precipitates. A quantity of urine was accordingly mixed with acetate of lead, and then with basic acetate of lead, until the latter gave no more precipitate. After the liquid had become clear, it was decanted and mixed with an excess of ammonia. The precipitate thereby produced was allowed to settle, filtered off, completely washed, and then treated with dilute sulphuric acid in the cold. The excess of the latter was removed by means of carbonate of lead, and the filtered liquid was evaporated as usual by a current of air. The residue left on evaporation was treated with cold water, and the liquid, after being filtered from the mixture of chloride of lead and extractive matter (*a*) left undissolved, was evaporated as before. The syrupy residue now left was well shaken with cold alcohol, which left a portion (*b*) undissolved. The filtered liquid was evaporated, and the residue having been dissolved in a little alcohol, the solution was mixed with a large quantity of ether, which made it milky and produced a copious syrup-like deposit (*c*). This was allowed to settle, and the ethereal liquid was poured off and evaporated. The residue left on evaporation was dissolved in cold alcohol, and to the solution there was added an alcoholic solution of acetate of lead, which produced a precipitate containing much chloride of lead. The addition of a little ammonia to the filtered liquid gave rise to a second precipitate, which was filtered off, washed, and prepared in the usual manner for analysis.

I. 0·8405 grm. gave 0·5000 grm. carbonic acid and 0·1240 grm. water.

1·1070 grm. gave 0·1595 grm. chloride of platinum and ammonium.

0·5575 grm. gave 0·5285 grm. sulphate of lead.

In 100 parts it contained therefore

C	16·22
H	1·63
N	0·90
O	11·50
PbO	69·75
	<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C	53·63
H	5·41
N	2·99
O	37·97
	<hr/> 100·00

This analysis yielded so large an amount of nitrogen and so little hydrogen and oxygen as compared with the preceding analyses, that it becomes difficult to bring it into harmony with the latter without having recourse to very improbable hypotheses. This discordance would, however, not be sufficient to justify the conclusion that the extractive matter soluble in ether contained in the precipitate with ammonia has a different composition from that prepared in the same way from the two other lead precipitates. By subsequent experiments it was indeed rendered very probable that they are identical in composition. It may therefore be inferred that in this case the discrepancy was due to some error, perhaps analytical. I should have hesitated in giving the details of this analysis had I not been desirous of presenting, without making any selection, the whole of the evidence on which my final conclusions are based.

The syrup-like deposit (c), insoluble in ether, was dissolved in alcohol. The solution was mixed with an alcoholic solution of acetate of lead, and the precipitate thereby produced was filtered off, washed with alcohol, and treated with dilute sulphuric acid. The excess of acid was removed by means of carbonate of lead, and the filtered liquid was evaporated in the air-current. The residue left on evaporation was treated with cold water, which left a quantity of chloride of lead undissolved; and to the filtered liquid there was added acetate of lead, and then a large quantity of alcohol. This gave a precipitate which, after being treated in the usual manner, was analyzed, the results obtained being as follows:—

- II. 1·1540 grm. gave 0·6320 grm. carbonic acid and 0·2050 grm. water.
 1·7405 grm. gave 0·2700 grm. chloride of platinum and ammonium.
 0·3005 grm. gave 0·2570 grm. sulphate of lead.
 0·7200 grm. gave 0·1015 grm. chloride of silver, equivalent to 0·0986 grm. chloride of lead.

In 100 parts it contained therefore

C	14·93
H	1·97
N	0·97
O	16·51
PbO	51·92
PbCl	13·70
	<hr/> 100·00

The substance combined with oxide and chloride of lead contained, in 100 parts,

C	43·43
H	5·73
N	2·82
O	48·02
	<hr/> 100·00

Several subsequent analyses led to the same composition as this. I shall therefore defer for the present giving the corresponding formula.

The syrupy matter, insoluble in cold alcohol (*b*), was treated with cold water, which left a quantity of gelatinous matter undissolved. Through the filtered liquid sulphuretted hydrogen was passed in order to precipitate the lead in solution, and after being again filtered it was evaporated in the air-current to a syrup, which was treated with cold alcohol as long as anything was dissolved. A portion (*d*) was left undissolved. To the filtered liquid there was added an alcoholic solution of acetate of lead, which produced a cream-coloured precipitate. This was filtered off and treated as usual before being analyzed.

III. 1·1430 grm. gave 0·5310 grm. carbonic acid and 0·1720 grm. water.

1·6965 grm. gave 0·1100 grm. chloride of platinum and ammonium.

0·6470 grm. gave 0·6245 grm. sulphate of lead.

1·0900 grm. gave 0·0485 grm. chloride of silver, equivalent to 0·0471 grm. chloride of lead.

In 100 parts it contained therefore

C	12·67
H	1·67
N	0·40
O	13·39
PbO	67·55
PbCl	4·32
	<hr/> 100·00

The substance combined with oxide and chloride of lead contained, in 100 parts,

C	45·04
H	5·94
N	1·44
O	47·58
	<hr/> 100·00

The portion of the syrupy residue (*d*) which was left undissolved by cold alcohol in the preparation of the preceding compound was dissolved in cold water, and to the solution acetate of lead and alcohol were added, which produced a dirty-white precipitate. This was filtered off, washed with alcohol, suspended in water, and decomposed with sulphuretted hydrogen. The filtered liquid was evaporated in the air-current, and the syrupy residue was treated with alcohol, which left a portion of it undissolved. The latter,

after the liquid had been poured off, was dissolved in water, and the solution mixed with acetate of lead and alcohol. The dirty-white precipitate thereby produced was filtered off, washed, dried, and analyzed.

IV. 0.5750 grm. gave 0.5355 grm. carbonic acid and 0.2010 grm. water.

0.7260 grm. gave 0.2595 grm. chloride of platinum and ammonium.

0.3585 grm. gave 0.1930 grm. sulphate of lead.

In 100 parts it contained therefore

C	25.39
H.....	3.88
N.....	2.24
O.....	28.91
PbO.....	39.58
	<hr/> 100.00

The substance combined with oxide of lead contained, in 100 parts,

C	42.02
H.....	6.42
N.....	3.70
O.....	47.86
	<hr/> 100.00

The results obtained in this analysis approximate to those yielded by Analysis II., Series D.

The mixture of chloride of lead and extractive matter (*a*) which was obtained in this series of experiments was also examined, and a lead compound was prepared from it which was submitted to analysis. The results, however, do not possess sufficient interest to make them worthy of communication. They served to show that the organic portion of the mixture did not differ in composition very widely from the extractive matters, the compounds of which had been previously analyzed.

It was about this time that I made a discovery of some interest connected with the chemistry of urine. I was occupied with the examination of the lead precipitate produced by ammonia in the liquid filtered from the precipitate with basic acetate of lead, and having treated it in the manner above described for the purpose of separating the extractive matters contained in it, I obtained an alcoholic solution of the latter, to which I added, as usual, a quantity of ether, and I observed after some time, mingled with the glutinous deposit produced by the ether, a quantity of crystalline matter. On pouring off the liquid and adding a little cold water to the deposit, the crystalline portion was left undissolved, and after filtration and washing had the appearance of a silky mass of a brownish hue. By dissolving it in boiling water and adding animal charcoal, the colour was removed; and on evaporating the filtered liquid I obtained a substance, crystallizing in white silky needles, which had the properties of tyrosine. It was very little soluble in cold water, and crystallized from the solution in

boiling water in snow-white masses consisting of star-shaped groups of needles. It gave, on being tried by Piria's method for discovering tyrosine, a very decided reaction. Its watery solution also gave, with nitrate of mercury, the reaction peculiar to tyrosine.

Among my collection of products from urine, there is one also belonging to this period; but by what means it was procured I cannot state, as I can find no memoranda relating to it among my notes. All that I can say regarding its preparation is, that it was obtained, like the other, from the lead precipitate with ammonia, and I think by a similar process. It is, however, totally different in its properties. It consists of regular, colourless crystals, and has a sweet taste. It dissolves in boiling water, but is not easily soluble in cold water. Its watery solution, on the addition of a salt of copper and an excess of caustic soda, turns blue; but no suboxide of copper is deposited on boiling the liquid. From its giving, when treated in the manner described by Scherer, with nitric acid, then with chloride of calcium and ammonia, the pink colour characteristic of inosite, I conclude that it consists of that peculiar species of sugar.

It is well known that both tyrosine and inosite are found in the urine in disease; and as the urine employed in my experiments was secreted by a great number of individuals, it seemed not improbable that among those individuals there might be some whose urine contained those bodies as the result of some morbid condition. In this case it would be the precipitates with basic acetate of lead and with ammonia in which these substances would be found, as they are neither of them precipitated by neutral acetate of lead. It is possible, however, that their occurrence may be due to some peculiar decomposition undergone by the extractive matters. I shall return to this point when I come to describe the properties of the latter.

F (1860).

The investigation had now reached a point at which, in my opinion, no advantage was to be derived from attempting to devise new methods of preparing the bodies under examination for analysis. I preferred going over the ground again, employing the same methods as before, and obtaining, if possible, some confirmation of the previous results.

The series of experiments now to be described consists of a renewed examination of the three lead precipitates in which the urinary extractive matters are contained—that produced in urine with neutral acetate of lead, that with basic acetate of lead in the liquid filtered from the first, and that with ammonia in the liquid filtered from the second precipitate.

The precipitate with acetate of lead was treated in the manner described in giving an account of the experiments of Series B. After being washed it was decomposed with sulphuric acid; the excess of the latter was removed from the filtered liquid by means of caustic baryta, and the phosphoric acid was precipitated by adding milk of lime. The filtered liquid having been mixed with an excess of acetic acid, acetate of lead and ammonia were

added to it, producing a cream-coloured precipitate. This was filtered off, washed with water, and treated with dilute sulphuric acid. The excess of the latter having been removed by means of carbonate of lead, the filtered liquid was evaporated in the air-current. The chloride of lead which was deposited during evaporation was filtered off, and the syrupy residue which was left at last was treated with cold alcohol. The liquid was poured off from the undissolved portion and evaporated, and the syrupy residue having been again dissolved in a little alcohol, the solution was mixed with a large quantity of ether, which threw down a portion of the matter in solution. The liquid, which was of a golden-yellow colour, was poured off from the insoluble deposit and evaporated to a syrup. This syrup was poured into a flask and agitated with a quantity of ether. After standing for some time, the ether, which had dissolved a portion of the syrup, was poured off and evaporated. The residue, which was free from compounds of chlorine, was dissolved in alcohol, and to the solution there was added an alcoholic solution of acetate of lead, which produced a precipitate of the usual colour. This was filtered off, washed with alcohol, dried *in vacuo*, and analyzed, the following results being obtained:—

I. 1·1080 grm. gave 0·9095 grm. carbonic acid and 0·2500 grm. water.

1·5635 grm. gave 0·1360 grm. chloride of platinum and ammonium.

0·7160 grm. gave 0·5430 grm. sulphate of lead.

These numbers lead to the following composition:—

C	22·38
H	2·50
N	0·54
O	18·78
PbO	55·80
		<hr/> 100·00

The composition of the substance combined with oxide of lead corresponds in this case with the formula $C_{86}H_{53}NO_{54}$, which requires

	Calculation.		Experiment.
C_{86}	516	50·83	50·65
H_{53}	53	5·22	5·67
N	14	1·37	1·22
O_{54}	432	42·58	42·46
	<hr/> 1015	<hr/> 100·00	<hr/> 100·00

On a previous occasion the analysis of the lead compound of the extractive matter soluble in ether led to the formula $C_{86}H_{51}NO_{52}$, which differs from the above by two equivalents of water. This difference might be ascribed to a more or less perfect desiccation of the lead compound; but I think it is more probably due to an absorption in one case of the elements of water, a process which often takes place with bodies of this class. From the extractive matter insoluble in ether but soluble in alcohol I also prepared a

lead compound, but the quantity obtained was not sufficient for a complete analysis.

The precipitate with basic acetate of lead was submitted to a process which did not differ from that just described, except in being rather simpler on account of the absence of phosphoric acid. It yielded by this treatment four substances; viz. one soluble in ether (*a*), a second soluble in alcohol but insoluble in ether (*b*), a third insoluble in a mixture of alcohol and ether (*c*), and a fourth insoluble in alcohol as well as in ether (*d*). The substance soluble in ether (*a*) was dissolved in absolute alcohol, and to the solution there was added an alcoholic solution of acetate of lead, which gave a precipitate of the usual colour. This was filtered off and prepared in the same manner as before for analysis.

II. 0.7215 grm. of this compound gave 0.6155 grm. carbonic acid and 0.1695 grm. water.

1.1295 grm. gave 0.1215 grm. chloride of platinum and ammonium.

0.4820 grm. gave 0.3680 grm. sulphate of lead.

In 100 parts it contained therefore

C	23.26
H	2.61
N	0.67
O	17.29
PbO	56.17
		<hr/> 100.00

The substance combined with oxide of lead contained, in 100 parts,

C	53.06
H	5.95
N	1.52
O	39.47
		<hr/> 100.00

The composition of the substance, combined in this case with oxide of lead, differs, as will be seen, very widely from that of the extractive matter soluble in ether as determined by previous experiments. This want of accordance is surprising, and I can only attribute it to some error in the analysis. The ratio between the nitrogen, the carbon, and the hydrogen is about the same as usual, and the discrepancy may therefore have arisen from an error in the estimation of the oxide of lead. There was not sufficient material left for another determination.

The substances (*b*) and (*c*), which I supposed to be essentially the same, were dissolved together in absolute alcohol. The solution was filtered from a small quantity of insoluble matter, and there was added to it an alcoholic solution of acetate of lead, which produced a precipitate of the usual colour. This was filtered off, washed, dried, and analyzed with the following results:—

III. 0.5855 grm. gave 0.4100 grm. carbonic acid and 0.1230 grm. water.
 0.7835 grm. gave 0.1625 grm. chloride of platinum and ammonium.
 0.4300 grm. gave 0.3265 grm. sulphate of lead.
 0.5085 grm. gave 0.0800 grm. chloride of silver, equivalent to 0.0777
 grm. chloride of lead.

These numbers lead to the following composition :—

C	19.09
H	2.33
N	1.30
O	18.39
PbO	43.60
PbCl	15.29
		<hr/> 100.00

The composition of the extractive matter contained in this lead compound corresponds with the formula $C_{38}H_{27}NO_{28}$, which requires

	Calculation.		Experiment.
C_{38}	228	46.24	46.44
H_{27}	27	5.47	5.66
N	14	2.83	3.16
O_{28}	224	45.46	44.74
	<hr/> 493	<hr/> 100.00	<hr/> 100.00

The substance (*d*) was treated with cold water, which dissolved the whole of it, with the exception of some chloride of lead which was filtered off. Sulphuretted hydrogen was passed through the liquid in order to precipitate the lead in solution; and having been again filtered, it was stirred in a mortar with sulphate of silver, by which means the hydrochloric acid as well as the excess of sulphuretted hydrogen contained in it were removed. Through the filtered liquid sulphuretted hydrogen was passed in order to precipitate the silver in solution. It was then filtered again, agitated with carbonate of lead in order to remove the sulphuric acid, filtered, freed from excess of lead by sulphuretted hydrogen, filtered again, and then evaporated in the air-current to a syrup. This syrup having been dissolved in water, acetate of lead was added to the solution, which after filtration was mixed with a large quantity of alcohol. This produced a precipitate, which was filtered off, washed with alcohol, suspended in water, and decomposed with sulphuretted hydrogen. The filtered liquid was evaporated in the air-current to a syrup, which was treated with cold alcohol until all the soluble matter was removed. The portion insoluble in alcohol* was dissolved in

* A part only of this was employed in the preparation of the lead compound. The remainder was kept in a flask for a considerable time. It was then found to have deposited a quantity of crystalline matter, which remained undissolved on the addition of cold water, but was soluble in boiling water. The boiling solution, after being decolorized with animal charcoal and filtered, deposited, on cooling, a quantity of tyrosine in white crystalline needles.

water, and to the solution acetate of lead was added, which gave a slight precipitate. The filtered liquid was mixed with a large quantity of alcohol, which produced a cream-coloured precipitate. This was filtered off and prepared in the usual manner for analysis.

IV. 1.0485 grm. gave 0.6810 grm. carbonic acid and 0.2195 grm. water.

1.5440 grm. gave 0.2755 grm. chloride of platinum and ammonium.

0.6120 grm. gave 0.4820 grm. sulphate of lead.

In 100 parts it contained therefore

C	17.71
H	2.32
N	1.12
O	20.90
PbO	57.95
		<hr/> 100.00

The substance combined with oxide of lead contained, in 100 parts,

C	42.11
H	5.51
N	2.66
O	49.72
		<hr/> 100.00

It will be seen that this analysis yielded numbers not differing very widely from those of Analysis II. Series D. The composition of the extractive matter combined with oxide of lead corresponds in both cases with the formula $C_{38}H_{29}NO_{34}$. A comparison of the two analyses affords also a further corroboration of what has been stated above, viz. that in the lead compounds containing chloride of lead, the latter replaces the same weight, not an equivalent quantity of oxide of lead. The difference between the amount of oxide of lead of the one compound and that of the chloride and oxide taken together of the other, is in this case greater than in those before referred to.

The precipitate produced by ammonia in the liquid filtered from that with basic acetate of lead still remained to be examined. In consequence, however, of a change of residence and other unforeseen circumstances the investigation suffered a lengthened interruption at this stage, and was only recommenced after an interval of two years. During this time the precipitate was kept in well-stoppered bottles covered with water, so as to preserve it moist and out of immediate contact with the atmosphere. It had undergone no perceptible change. It was therefore filtered off and treated with dilute sulphuric acid. The excess of acid was removed by means of carbonate of lead, and the filtered liquid was evaporated at a moderate temperature in a hot-air stove instead of, as hitherto, by means of a current of air, which I now no longer had the means of producing of the requisite strength. The residue left after evaporation was treated with cold water, which left a quantity of chloride of lead of a dirty-yellow colour undis-

solved. The liquid was filtered, sulphuretted hydrogen was passed through it, and after being filtered from the precipitated sulphide of lead, a boiling solution of sulphate of silver was added to it as long as any precipitate of sulphide or chloride of silver was produced. The excess of silver was removed from the filtered liquid by sulphuretted hydrogen, and the liquid having been again filtered was agitated with carbonate of lead, which took away the sulphuretted hydrogen and the sulphuric acid contained in it. It was then filtered, sulphuretted hydrogen was passed through it, and after being filtered from the sulphide of lead, it was evaporated in the hot-air stove to a syrup. This syrup was then poured into a flask, a little alcohol and a large quantity of ether were added, and the whole was well shaken. After standing for some time the ethereal liquid was poured off from the undissolved portion of the syrup (*e*) and evaporated. The residue left after evaporation was treated with water, and the resulting solution was filtered from a little fatty matter which was left undissolved and evaporated. The residue was poured into a flask and treated with ether. After standing some time the liquid was poured off from the undissolved syrup-like matter (*f*) and evaporated, and the residue was treated again with ether, which left a little more syrup-like matter undissolved. The residue now left was dissolved in alcohol, and to the solution acetate of lead and ammonia were added. The precipitate thereby produced was filtered off, washed with alcohol, and treated with a little dilute acetic acid, in which it was for the most part soluble. Some brown flocks which were left undissolved were filtered off, and the liquid was mixed with a large quantity of alcohol which produced a pale cream-coloured precipitate. This was filtered off, washed, dried, and analyzed, the following results being obtained:—

V. 1·2335 grm. gave 0·8460 grm. carbonic acid and 0·2375 grm. water.

1·5440 grm. gave 0·1175 grm. chloride of platinum and ammonium.

0·7570 grm. gave 0·6265 grm. sulphate of lead.

These numbers lead to the following composition:—

C	18·70
H	2·13
N	0·47
O	17·81
PbO	60·89
		<hr/>
		100·00

The composition of the substance combined with oxide of lead agrees tolerably well with the formula $C_{86}H_{59}NO_{60}$, which requires

	Calculation.		Experiment.
C_{86}	516	48·27	47·81
H_{59}	59	5·52	5·44
N	14	1·30	1·20
O_{60}	480	44·91	45·55
	<hr/> 1069	<hr/> 100·00	<hr/> 100·00

This formula represents a substance containing several atoms more water than the extractive matter soluble in ether, the composition of which was arrived at by Analysis III. Series A. The tendency to absorption of water by this body was already indicated by Analysis I. of the present series. Here it has taken place to a still greater extent, and has probably attained the extreme possible limit. The absorption of water in this instance I was at the time inclined to attribute to the evaporation of the solutions containing the extractive matters having been conducted at a higher temperature than before. I now think it more probable that it was due to the length of time during which the lead precipitate with ammonia was kept in contact with water.

The syrup-like matter insoluble in alcohol and ether (*e*) and that insoluble in ether alone (*f*) were mixed together and treated with alcohol. After standing for some time the liquid was poured off from some glutinous substance (*g*), which was left undissolved, and evaporated. The residue left on evaporation was treated with warm absolute alcohol, which dissolved the greatest part of it. To the solution there was added a little acetate of lead, and after being filtered from the precipitate it gave, with an excess of alcoholic lead solution, an abundant precipitate, which after being treated in the usual manner was analyzed.

VI. 0.8800 grm. of this precipitate gave 0.7235 grm. carbonic acid and 0.2335 grm. water.

1.4885 grm. gave 0.3300 grm. chloride of platinum and ammonium.

0.2160 grm. gave 0.1415 grm. sulphate of lead.

In 100 parts it contained therefore

C	22.42
H	2.94
N	1.39
O	25.05
PbO	48.20
		<hr/> 100.00

The substance combined with oxide of lead contained, in 100 parts,

C	43.28
H	5.69
N	2.68
O	48.35
		<hr/> 100.00

The glutinous substance (*g*) left undissolved by alcohol was treated with water, and the resulting dark-brown solution was filtered from some undissolved gelatinous matter and agitated with oxide of mercury, which took up a great deal of the colour, leaving the solution of a pale yellow tint. After filtration sulphuretted hydrogen was passed through it in order to precipitate the mercury in solution, and after being again filtered it was evaporated, when it left a pale yellow amorphous residue. This was treated

with alcohol, and the portion left undissolved by the alcohol was dissolved in water. To the watery solution acetate of lead was added, and the filtered liquid was mixed with a large quantity of alcohol, which produced a pale cream-coloured precipitate. This was filtered off and prepared in the usual manner for analysis.

VII. 1·0625 grm. of this precipitate gave 0·7060 grm. carbonic acid and 0·2400 grm. water.

2·0080 grms. gave 0·2505 grm. chloride of platinum and ammonium.

0·8545 grm. gave 0·6210 grm. sulphate of lead.

In 100 parts it contained therefore

C	18·12
H	2·50
N	0·78
O	25·13
PbO	53·47
		<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C	38·94
H	5·40
N	1·68
O	53·98
		<hr/> 100·00

These numbers seem to point to the conclusion that the extractive matter insoluble in alcohol had also absorbed a considerable quantity of water, in consequence of the long-continued contact of the mixed lead compounds with water. As there seemed, however, some reason to suspect the presence of glucose in the compound of this analysis, I thought it hardly worth while to devise any formula to represent its composition.

G (1862).

The next series of experiments was made with urine obtained from the surgical wards of the Manchester Infirmary. By employing material derived from an entirely different source, I hoped to obtain further confirmation of the results previously arrived at. I refrained, however, from using any truly morbid urine for fear of introducing an element of uncertainty into the experiments. The urine supplied to me, through the kindness of the medical officers of the Institution, did not differ perceptibly from that of healthy individuals, the appearance, colour, and reaction being quite normal.

The urine was first mixed with a solution of acetate of lead, and the precipitate thereby produced was separated and treated in the manner before described, for the purpose of getting rid of the phosphoric and uric acid contained in it; and by means of acetate of lead and ammonia a precipitate was then obtained containing, besides chloride of lead, only compounds of

the extractive matters. This was then added to the lead precipitate produced by ammonia in the urine after being filtered from the precipitate with acetate of lead. The mixture contained, therefore, the extractive matters of the three lead precipitates, which had before been separately examined. It was treated in the manner described near the conclusion of the preceding section, that is to say, it was acted on by dilute sulphuric acid; the hydrochloric acid set at liberty was removed by means of sulphate of silver, the excess of silver was precipitated as sulphide, the sulphuric acid was got rid of with carbonate of lead, and the lead in solution having been precipitated with sulphuretted hydrogen, the liquid was evaporated, leaving a residue containing the extractive matters, which were separated from one another by means of alcohol and ether. In this way I obtained a substance soluble in alcohol and ether (*a*), a second soluble in alcohol but insoluble in ether (*b*), and a third insoluble in alcohol and ether (*c*). From the first a lead compound was prepared in the usual manner, the analysis of which led to the following results:—

I. 0.9530 grm. gave 0.6695 grm. carbonic acid and 0.1665 grm. water.

1.3260 grm. gave 0.0995 grm. chloride of platinum and ammonium.

0.5250 grm. gave 0.4500 grm. sulphate of lead.

In 100 parts it contained therefore

C	19.15
H	1.94
N	0.47
O	15.37
PbO	63.07
	<hr/>
	100.00

The substance combined with oxide of lead contained, in 100 parts,

C	51.88
H	5.25
N	1.27
O	41.60
	<hr/>
	100.00

It will be seen that the composition of the extractive matter of this compound may be expressed by the formula $C_{86}H_{51}NO_{52}$, the same to which Analysis III. Series A led.

The substance *b* was treated in the cold with absolute alcohol, which left a portion of it undissolved. The solution was evaporated, and the residue was treated as before with absolute alcohol. The residue left after the second evaporation was dissolved in water. Acetate of lead was added to the solution, and the filtered liquid was mixed with alcohol, which produced a precipitate. This was treated as usual and then analyzed.

II. 1.0980 grm. gave 0.7935 grm. carbonic acid and 0.2410 grm. water.

1.6135 grm. gave 0.2740 grm. chloride of platinum and ammonium.

0.4765 grm. gave 0.3550 grm. sulphate of lead.

These numbers lead to the following composition:—

C	19·70
H.....	2·43
N.....	1·06
O	22·00
PbO.....	54·81
	<hr/> 100·00

The composition of the substance combined with oxide of lead agrees very well with the formula $C_{38}H_{27}NO_{32}$, which requires

	Calculation.		Experiment.
C_{38}	228	43·42	43·59
H_{27}	27	5·12	5·37
N	14	2·66	2·34
O_{32}	256	48·80	48·70
	<hr/> 525	<hr/> 100·00	<hr/> 100·00

Several of the preceding analyses gave a composition corresponding more or less closely with the same formula, as I shall show when I come to give a summary of the whole of the results obtained.

The substance *c*, which was insoluble in alcohol, was treated with cold water. The resulting solution was filtered from some gelatinous matter (consisting of silica), which was left undissolved, and then agitated with oxide of mercury. After filtration, sulphuretted hydrogen was passed through it in order to precipitate the mercury in solution, and after being filtered it was evaporated. The glutinous residue left on evaporation was dissolved in a very small quantity of water, and the solution was mixed with a large quantity of alcohol, which precipitated a substance of a glutinous nature. After standing for some time the liquid was poured off, and the precipitated matter was washed with alcohol and then dissolved in a little water. To the solution there was added acetate of lead, which produced a dirty-yellow precipitate; and the addition of a considerable quantity of alcohol to the filtered liquid gave rise to a second precipitate, which was filtered off, washed, dried, and analyzed as usual.

III. 1·1780 grm. of this precipitate gave 0·8090 grm. carbonic acid and 0·2660 grm. water.

2·0705 grms. gave 0·3585 grm. chloride of platinum and ammonium.

0·7485 grm. gave 0·5595 grm. sulphate of lead.

In 100 parts it contained therefore

C	18·72
H.....	2·50
N.....	1·08
O	22·70
PbO.....	55·00
	<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C	41·60
H	5·55
N	2·40
O	50·45
	<hr/> 100·00

Two of the preceding determinations, viz. Analysis II. Series D and Analysis IV. Series F, led to nearly the same results, the composition of the substance contained in the lead compound corresponding in all three cases with the formula $C_{38}H_{29}NO_{34}$.

Being curious to ascertain what had been taken up by the oxide of mercury which was employed in the purification of the substance used in the preparation of the last lead compound, it was, after being washed, suspended in water and decomposed by a current of sulphuretted hydrogen. The filtered liquid was evaporated to a syrup. This syrup, which was very brown, was dissolved again in a very little water, and the solution was mixed with a large quantity of alcohol, which produced a glutinous deposit. The latter, after the liquid had been poured off, was dissolved in a little water, and to the solution there was added acetate of lead, which gave a dirty-yellow precipitate. The filtered liquid was mixed with a large quantity of alcohol, and the resulting precipitate was filtered off, washed, dried, and analyzed.

0·7170 grm. of this precipitate gave 0·5365 grm. carbonic acid and 0·1650 grm. water.

1·0915 grm. gave 0·6240 grm. chloride of platinum and ammonium.

0·3615 grm. gave 0·2595 grm. sulphate of lead.

In 100 parts it contained therefore

C	20·40
H	2·55
N	3·59
O	20·65
PbO	52·81
	<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C	43·24
H	5·41
N	7·60
O	43·75
	<hr/> 100·00

From the unusually large amount of nitrogen yielded by this analysis, it must be concluded that the oxide of mercury took up some substance differing in composition from the extractive matters, probably a product of decomposition of the latter.

This series of experiments confirms in a remarkable manner the results

previously obtained, and serves to show that the composition of the extractive matters does not vary with the source whence they are derived.

H (1863).

In order to remove any doubt that might still have remained in regard to the composition of the urinary extractive matters, I made another series of experiments, employing for this purpose ordinary healthy urine. The urine was mixed as usual with acetate of lead, and then with basic acetate, but the precipitate with the latter was alone made use of. This, after being washed with water, was treated in the manner before described, and yielded as usual a substance soluble in ether (*a*), a second soluble in alcohol but insoluble in ether (*b*), a third insoluble in a mixture of alcohol and ether (*c*), and a fourth insoluble both in alcohol and in ether (*d*). The first of these was treated with water, which left a quantity of fatty matter undissolved. To the filtered liquid there was added acetate of lead; and having been again filtered, it was mixed with a large quantity of alcohol, which produced a precipitate of the usual appearance. The analysis of this precipitate yielded the following results:—

I. 1.0945 grm. gave 0.8540 grm. carbonic acid and 0.2115 grm. water.

1.5585 grm. gave 0.1265 grm. chloride of platinum and ammonium.

0.6600 grm. gave 0.5255 grm. sulphate of lead.

In 100 parts it contained therefore

C	21.28
H	2.14
N	0.50
O	17.50
PbO	58.58
	<hr/> 100.00

The substance combined with oxide of lead contained, in 100 parts,

C	51.37
H	5.16
N	1.20
O	42.27
	<hr/> 100.00

If this composition be compared with that yielded by Analysis III. Series A, it will be seen that the difference between them is not greater, even as regards the amount of oxide of lead, than is usually found in two analyses of the same substance. This near approximation in the results of the first and the concluding series of experiments, as far as regards one of the extractive matters, is remarkable.

The substances *b* and *c*, though probably one and the same, were examined separately, in order to remove all doubt as to the identity of their composition. The former was treated with absolute alcohol, in which it was entirely soluble. To the solution there was added a little acetate of lead, and the dark-brown precipitate produced by the latter having been

filtered off, an excess of alcoholic-lead solution gave an abundant precipitate, which was filtered off and then treated with acetic acid, less acid being taken than would have sufficed to dissolve it entirely. The filtered liquid was mixed with a large quantity of alcohol, which gave a precipitate of a pure cream-colour. This was analyzed in the usual manner.

II. 1.1365 grm. gave 0.9295 grm. carbonic acid and 0.2695 grm. water.

1.5655 grm. gave 0.2595 grm. chloride of platinum and ammonium.

0.6855 grm. gave 0.4585 grm. sulphate of lead.

In 100 parts it contained therefore

C	22.30
H.....	2.63
N.....	1.04
O.....	24.82
PbO.....	49.21
	<hr/>
	100.00

The substance combined with oxide of lead contained, in 100 parts,

C.....	43.90
H.....	5.15
N.....	2.04
O.....	48.91
	<hr/>
	100.00

The substance *c* was treated with alcohol. The liquid was poured off from the portion left undissolved and evaporated. The residue was dissolved in a little alcohol, and the solution was mixed with a large quantity of ether, which precipitated a syrupy mass. After the latter had settled, the liquid was poured off, and the syrup was dissolved in water. Acetate of lead was added to the solution, and the filtered liquid was mixed with a quantity of alcohol. The precipitate thereby produced was filtered off, washed, dried, and analyzed, the results being as follows:—

III. 1.1475 grm. gave 0.8800 grm. carbonic acid and 0.2610 grm. water.

1.5390 grm. gave 0.2995 grm. chloride of platinum and ammonium.

0.7300 grm. gave 0.5135 grm. sulphate of lead.

In 100 parts it contained therefore

C.....	20.91
H.....	2.52
N.....	1.22
O.....	23.60
PbO.....	51.75
	<hr/>
	100.00

The substance combined with oxide of lead contained, in 100 parts,

C.....	43.33
H.....	5.22
N.....	2.52
O.....	48.93
	<hr/>
	100.00

Hence it follows that the substances *b* and *c* have the same composition, a composition corresponding with the formula $C_{38}H_{27}NO_{32}$, the same to which Analysis II. of the preceding series conducted.

The substance *d* was treated with cold water. The resulting dark-brown solution was filtered from some gelatinous matter, which remained undissolved and was found to consist chiefly of silica, and then mixed with an excess of acetate of lead. This produced a dark-brown precipitate, which was filtered off. A little ammonia and a large quantity of alcohol were then added to the liquid, and the bulky cream-coloured precipitate produced was filtered off, washed with alcohol, suspended in water, and decomposed with sulphuretted hydrogen. The filtered liquid was evaporated, and the brown glutinous residue which was left was dissolved in water. The addition of acetate of lead to the solution produced a brown precipitate. The filtered liquid gave, on being mixed with alcohol, a cream-coloured precipitate, which was filtered off, washed, dried, and analyzed as usual.

IV. 1·1760 grm. gave 0·9445 grm. carbonic acid and 0·3165 grm. water.

1·6280 grm. gave 0·5300 grm. chloride of platinum and ammonium.

0·6715 grm. gave 0·4330 grm. sulphate of lead.

In 100 parts it contained therefore

C.....	21·90
H	2·99
N	2·04
O.....	25·63
PbO.....	47·44
	<hr/> 100·00

The substance combined with oxide of lead contained, in 100 parts,

C.....	41·66
H.....	5·68
N.....	3·88
O.....	47·78
	<hr/> 100·00

The unusually large amount of nitrogen yielded by this analysis was probably due to an admixture of the impurity which in the former experiments was removed by means of oxide of mercury, and which contains, as I have shown, more than seven per cent. of that element. Had I employed the same method of purification as before, the composition would probably have corresponded more closely with that to which Analysis III. of the preceding series and several previous determinations led, and which may be represented by the formula $C_{38}H_{29}NO_{34}$.

The numerical results obtained by these determinations are, I think, sufficiently numerous and concordant to allow of definite conclusions being drawn regarding the composition of the urinary extractive matters.